# Acquisition techniques for measuring GABA using single voxel proton magnetic resonance spectroscopy in the human brain in vivo at 7T

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*In the memory of my father, Dr. Seyedmojtaba Rouhani Rankouhi*

**CONTENTS**

# Chapter 1-Introduction

## Introduction

There is currently great interest in the field of neuroscience to measure gamma-aminobutyric acid (GABA) in the healthy and diseased human brain *in vivo*, with the aim of studying the inhibitory role of this neurotransmitter in the functioning brain [1].

GABA is an inhibitory neurotransmitter with a low concentration of around 1-2 mMol/Kg in the human brain [2]. The only current method for measuring GABA *in vivo* is Magnetic Resonance Spectroscopy (MRS). MRS is a NMR based method to detect metabolites in a sample and currently is an established method for measuring the concentration of different metabolites in the human brain noninvasively in vivo. Compared to MRI, MRS provides physiological information rather than anatomical. Among different MR spectroscopy methods, two of the most common are single voxel spectroscopy (SVS) and chemical shift imaging (CSI) also known as magnetic resonance spectroscopy imaging (MRSI). SVS provides quantitative information from a single voxel while CSI provides this information from a much larger volume of interest consisting of multiple voxels. In this thesis we concentrate on proton magnetic resonance SVS approaches for measuring all metabolites in the human brain generally, and GABA specifically. The resonance frequency of a proton in addition to being dependent on its gyromagnetic ratio and the main magnetic field strength, is also dependent on its molecular environment mainly through shielding effects by surrounding electrons. If there was no shielding effect by the electrons, the magnetic resonance spectrum would only present a single peak. Hence, peaks arising at different frequencies in a NMR spectrum are used as probe of the chemical environment and are utilized to measure concentrations of different metabolites in the voxel.

To perform a SVS acquisition, some preliminary procedures are necessary. Anatomical images are acquired to be used as references for positioning the voxel of interest for SVS. Examples for this are MPRAGE [3] or MP2RAGE [4] sequences used to obtain T1-weighted anatomical images. Also, $B_0$ shimming of the voxel of interest is necessary. In fact, a homogeneous field is crucial to obtain a good quality spectrum from the voxel of interest. A homogeneous local field

is obtained using $B_0$ shimming prior to the spectroscopy. An example of a $B_0$ shimming method is FASTESTMAP [5]. In regions of the brain where bone, fat or air is close, it is very difficult to get a good B0 homogeneity because of large tissue magnetic susceptibility differences which makes shimming of the volume of interest very difficult.

## Single voxel localization

SVS and MRSI are localized spectroscopy techniques. The main aim of localization is to acquire signal from a selected volume with minimum possible partial volume effects or contribution of signals from outside of the volume of interest. Localization also provides the possibility of attaining better $B_0$ and $B_1$ homogeneity in the selected voxel which would provide narrower linewidth of the acquired spectra from the volume of interest and more exact flip angle of the RF pulses over the volume of interest. Currently, a number of different localization techniques are available. Here, these pulse sequences, their main properties, advantages and disadvantages are briefly discussed. Single voxel spectroscopy generally selects a voxel by selection of three orthogonal slices. This is achieved by applying an RF pulse and a slice selection gradient field simultaneously in a certain direction. Various SVS localization techniques have been introduced each having advantages and drawbacks:

- STEAM (STimulated Echo Acquisition Mode)

  This sequence consists of three 90° pulses. The echo collected is a stimulated echo. All other echoes or FIDs are suppressed using spoiler gradients that are properly placed in the timing intervals between RF pulses. An advantage is that it is possible with this sequence to achieve ultrashort TE mainly because the time between the second and third RF pulses which is called mixing time TM the magnetization stays in longitudinal direction and hence will decay only with T1 relaxation. A disadvantage is that the generated echo has half the intensity of a spin echo. The STEAM sequence introduced by Frahm et al is shown in Figure 1[6].

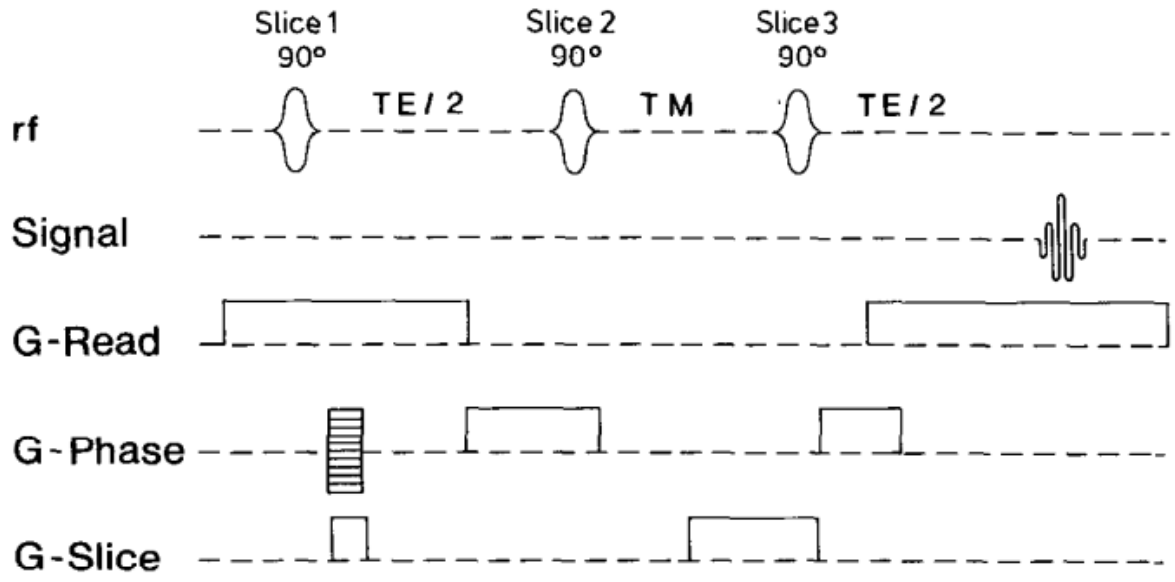



*Figure 1: STEAM sequence. taken with permission from [6].*

- PRESS (Point RESolved Spectroscopy)

  This sequence consists of a 90° excitation pulse and two 180° refocusing pulses. An advantage of PRESS sequence compared with STEAM is that it generates a full intensity echo. The PRESS sequence introduced by Bottomley is presented in Figure 2 [7].

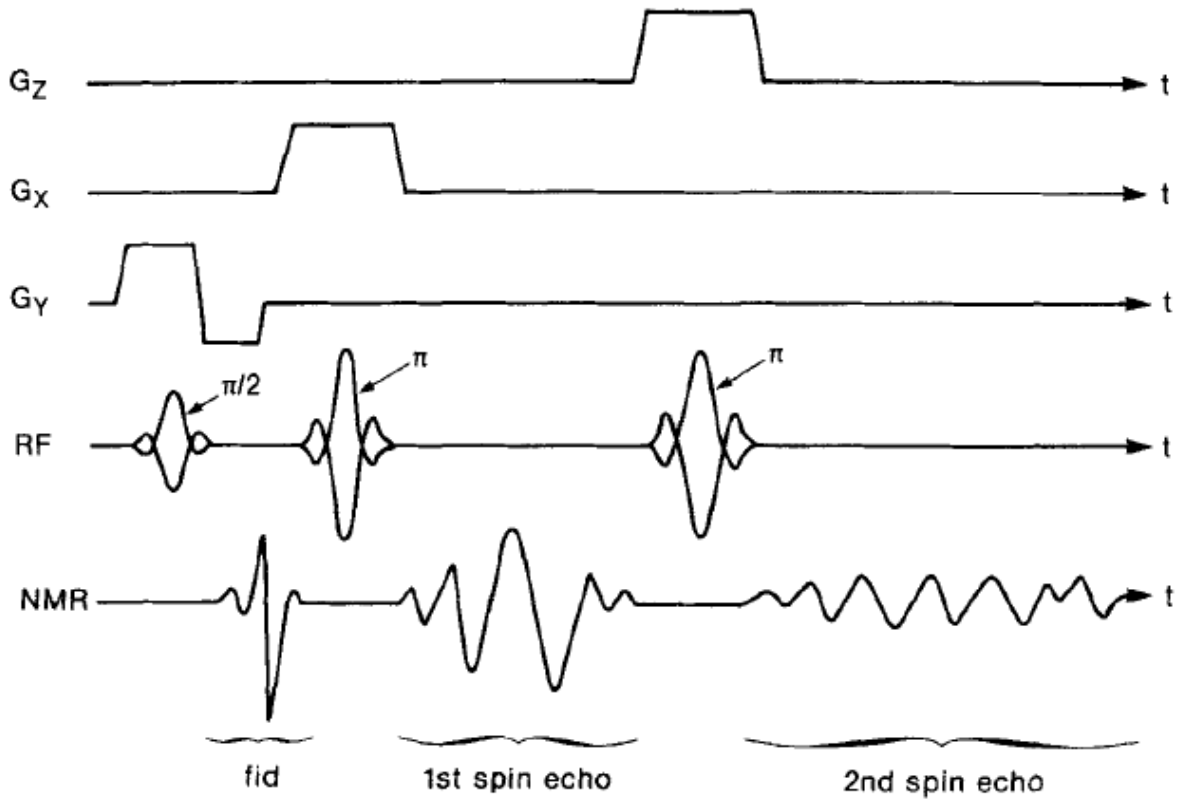



*Figure 2: PRESS sequence, taken with permission from [7].*

- SPECIAL (SPin ECho, full Intensity Acquired Localized spectroscopy)

  There is a pre-inversion in two modes of ON and OFF in this sequence, where a slice either is or is not inverted. The two other slices are selected by a spin echo. The voxel is selected by subtracting ON and OFF modes. As only two RF pulses in this sequence generate the echo, it is possible to achieve an ultrashort TE acquisition with this sequence. Also, the sequence generates a full intensity echo which is another advantage. The subtraction basis of the method brings the danger of its sensitivity to motion. The SPECIAL sequence introduced by Mlynarik et al is presented in Figure 3 [8].

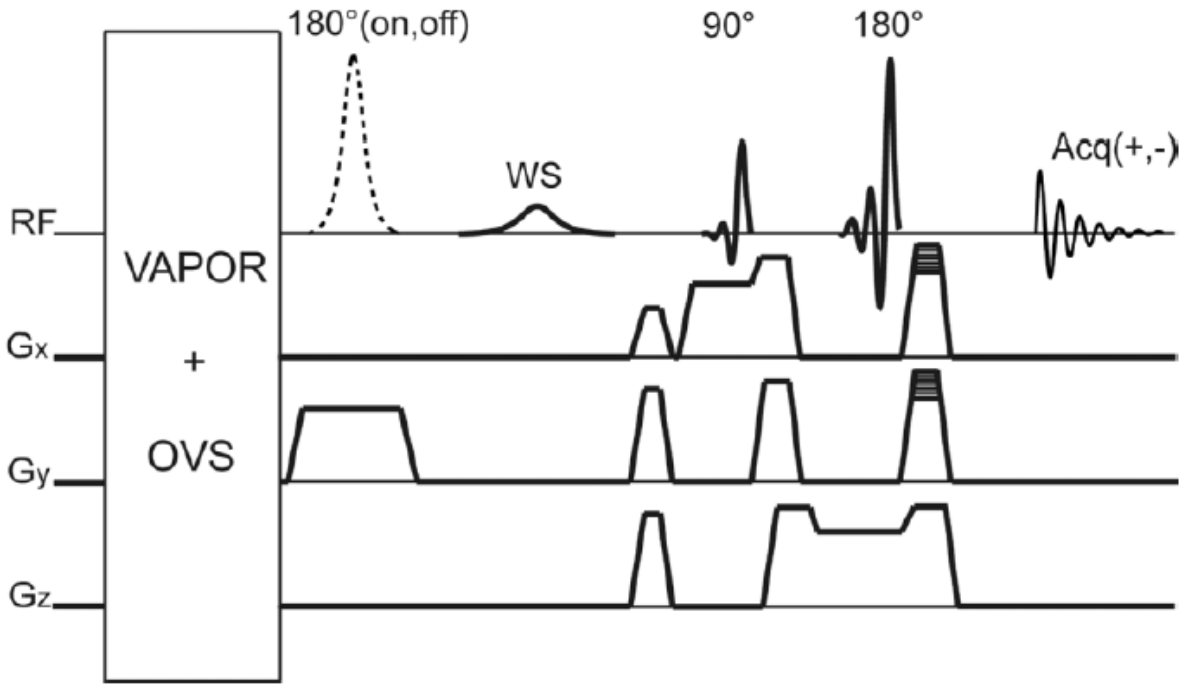



*Figure 3: SPECIAL sequence, taken with permission from [8].*

- SADLOVE (Single-shot ADiabatic LOcalized Volume Excitation) and LASER (Localization by Adiabatic SElective Refocusing)

  These two sequences include one adiabatic 90° pulse and six adiabatic refocusing pulses. A disadvantage of SADLOVE [9] or LASER [10] sequences is their relatively longer minimum achievable TE because of having seven RF pulses compared with PRESS or STEAM which each includes just three RF pulses or SPECIAL which effectively needs only two RF pulses to generate an echo. On the other hand, SADLOVE and LASER are largely immune to B1 variations because of having all adiabatic RF pulses which, is a beneficial characteristic at ultrahigh field where the B1 inhomogeneity is higher. Indeed, adiabatic pulses are class of RF pulses that can excite, refocus or invert magnetization uniformly even in the presence of a spatially nonuniform B1 field. In fact, as long as the amplitude of the B1 exceeds a threshold, spins that experience different B1 fields can be excited with the same flip angle. Therefore, adiabatic pulses are robust to variation in B1. Also, since the adiabatic pulses have generally much wider bandwidth, chemical shift displacement error (CSDE) is low to negligible

when SADLOVE or LASER is used for SVS. The two sequences are presented in Figures 4 and 5 respectively.

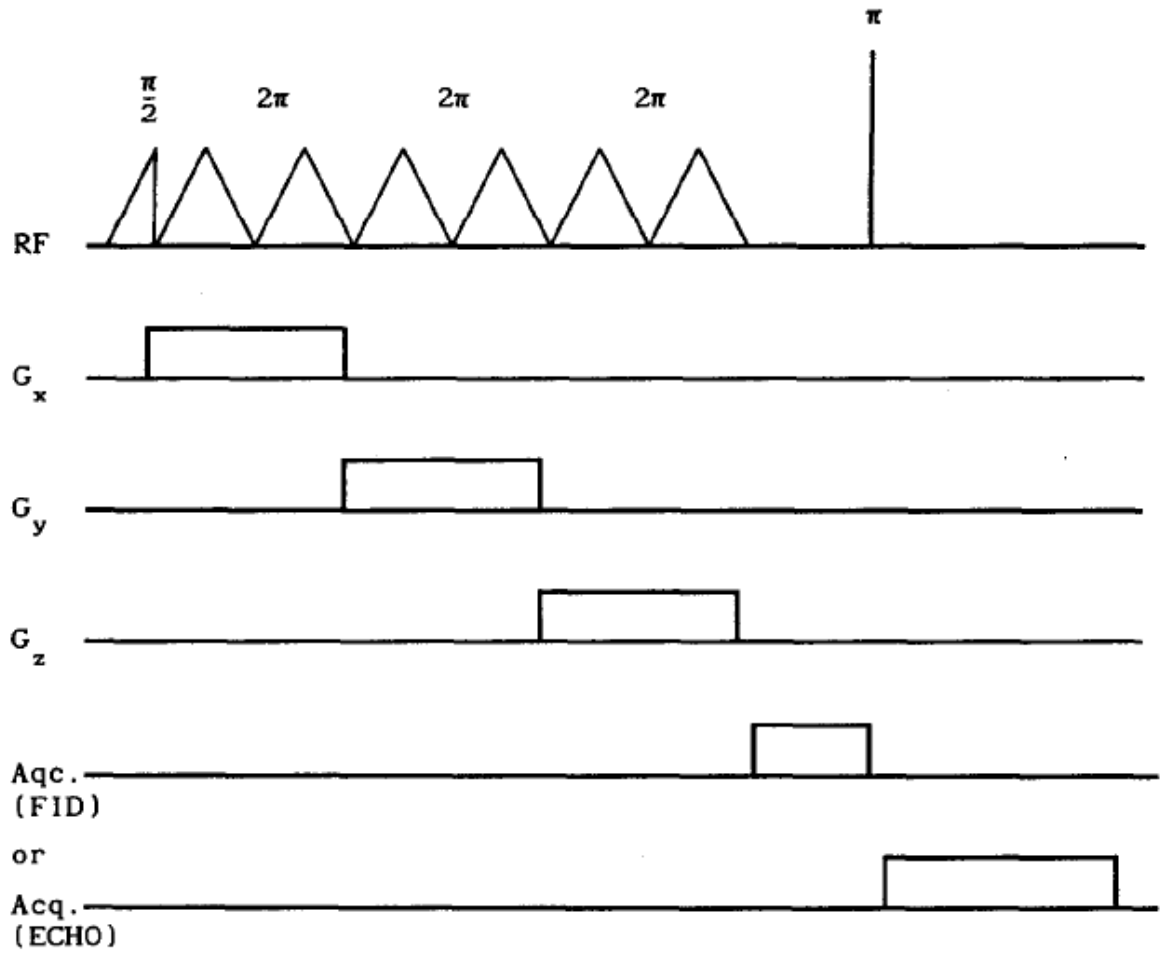



*Figure 4: SADLOVE sequence, taken with permission from [11]*

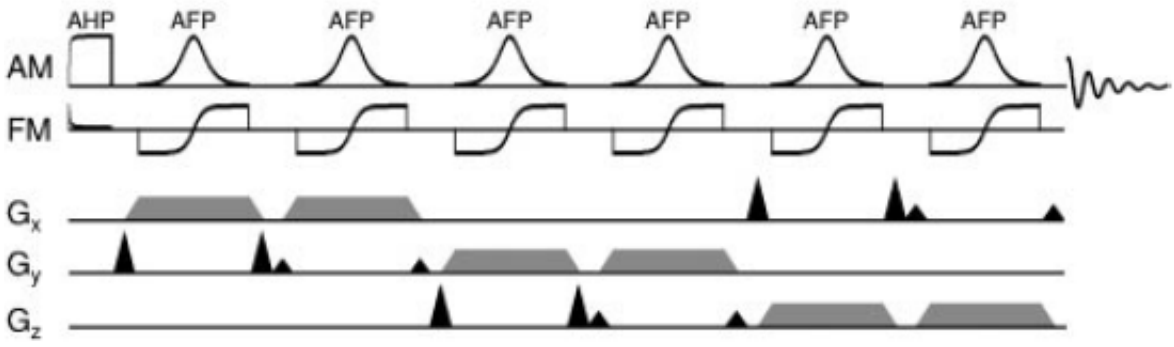



*Figure 5: LASER sequence, taken with permission from [10]*

- sLASER

  This sequence lands in between PRESS and LASER by providing relatively shorter minimum possible TE than LASER while having relatively lower sensitivity to B1 variations compared with PRESS or STEAM. The sequence consists of a non-adiabatic excitation pulse and two pairs of adiabatic refocusing pulses. The sLASER sequence introduced by Scheenen et al [12] is presented in Figure 6.

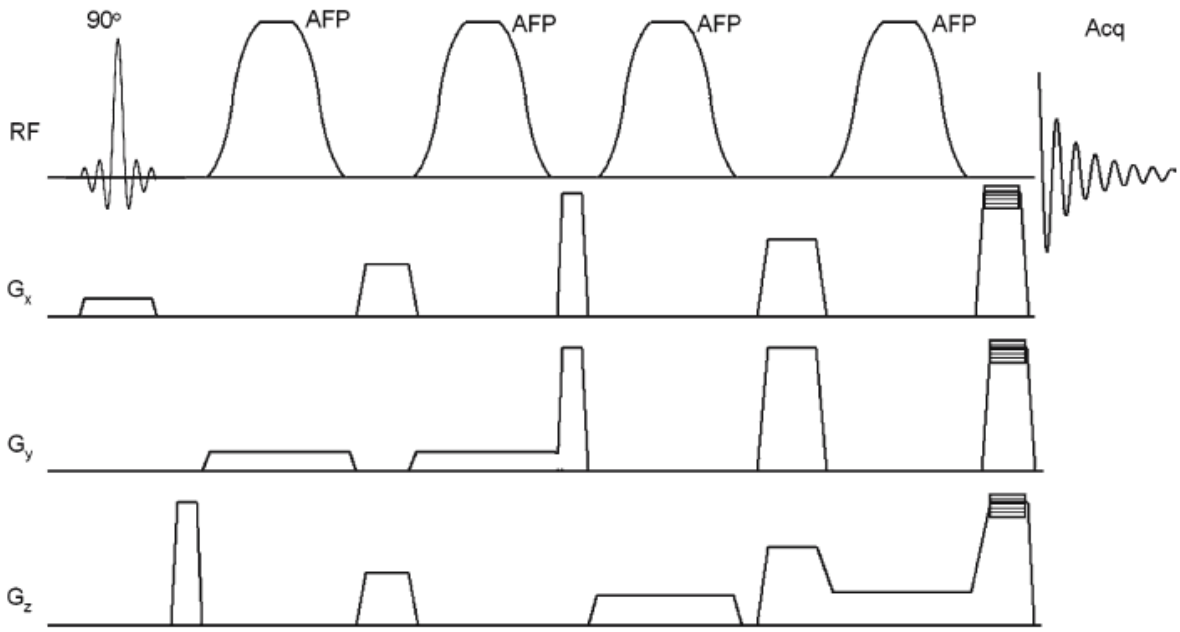



*Figure 6: sLASER sequence [12]*

## Water suppression

Water suppression is another component of the MR spectroscopy pulse sequence which is applied either prior to the localization block or is inserted within the localization block. The human body mostly consists of water. Thus, MRS of a voxel leads to a very large peak at water frequency and therefore, the metabolites which have much lower concentration than water will not be observable in a non-water-suppressed spectrum. A step crucial for MR spectroscopy is therefore water suppression. A combination of RF pulses and magnetic field gradients as a block of water suppression is hence necessary to be added prior to the localization block of the NMR sequence like WET [13] and VAPOR [14] or merged inside the

localization block like WATERGATE [15] and MEGA [16]. Particularly, WET water suppression (water suppression enhanced through T1 effects) which is used in the pulse sequences implemented in this thesis was developed from Bloch equation analysis of the longitudinal magnetization over ranges of T1 and B1, and provides T1 and B1 insensitive water suppression with use of four RF pulses which possess numerically optimized flip angles [13].

## J-coupling

Coupling is a magnetic interaction between nuclei with non-zero spin. There are two kinds of couplings: the direct interaction which is also called dipolar-dipolar coupling and the indirect interaction which is also called spin-spin coupling or J coupling or scalar coupling. The dipolar-dipolar coupling is around 1000 times larger than J coupling. In isotropic mobile liquids dipolar coupling is averaged out because of random motion of the molecules. The J coupling however is a through-bond magnetic interaction. The spin of a nucleus polarizes the electron spins and these polarized electrons change the energy level of the neighboring nuclei. This is reflected as a slightly increased or decreased energy level of the neighboring nucleus. This phenomenon is called J-coupling. This occurring energy level difference is independent of the strength of the main magnetic field. Since energy is proportional to frequency via Planck's constant, this energy level difference manifests as a change in frequency, and is represented as "J-coupling constant" (normally given in Hz). The value of the J-coupling constant depends on the strength of the chemical bond if it is a single, dual or triple bond. Also, it depends on the angles between the bonded atoms. The J-coupling gets weaker as the distance between the nuclei increases. The energy difference between two energy states of a spin (α and β) causes a singlet spectrum. With J coupling however each energy state splits to two, three, or more states which causes the spectrum appearing as doublet, triplet, quadruplet, etc. J coupling also causes different pattern of J coupled peaks at different echo times because of phase evolution by time, a concept which is utilized in J-editing methods.

## Chemical Shift Displacement Error (CSDE)

The precessional frequency of each proton ω as a function of position, given a Larmor frequency of $\omega_0$ in the presence of a linear constant gradient in certain direction $G_x$ is given by:

$$\omega(x) = \omega_0 + \gamma x G_x$$

Therefore, we have:

$$x = \frac{(\omega(x) - \omega_0)}{\gamma G_x}$$

Which means protons with different Larmor frequencies will be mapped in different positions, a phenomenon that is called chemical shift displacement error (CSDE). At higher fields, there is higher frequency separation between the spectral components (lines) which therefore leads to higher CSDE. In other words, CSDE means that single voxel spectroscopy selects a continuous sequence of voxels each representing a certain frequency in the spectrum. CSDE in each direction gets lower when the slice selection gradient in the corresponding direction gets higher. CSDE is also dependent on the bandwidth of the RF pulses that are used for localizing the single voxel. Conventional RF pulses that are used in STEAM, PRESS or SPECIAL sequences have relatively low bandwidths and therefore, these localization techniques suffer from a high CSDE at ultrahigh field. Adiabatic pulses have generally higher bandwidth and therefore are good candidates to tackle the problem of CSDE at ultrahigh field. Hence, sLASER and LASER sequences are more appropriate for SVS at ultrahigh field, offering low to negligible CSDE depending on the bandwidth of the adiabatic pulses used in these two sequences. For example, GOIA pulses are a class of adiabatic pulses that offer very wide bandwidth in the range of 20 KHz [17] which makes CSDE negligible.

## Advantages and disadvantages of ultrahigh field

The SVS methods that are introduced, implemented or investigated in this thesis have been performed on a human research 7T MR scanner; a main magnetic field strength which is considered as ultrahigh field. Measuring at ultrahigh field brings disadvantages and advantages with it. At ultrahigh field like 7T and above, the wavelength corresponding to the Larmor frequency becomes comparable with the diameter of the head. This fact causes a B1 drop in peripheral regions to the isocenter and eventually causes signal drop out and destructive changes in the image contrast [18]. Besides, variation of $B_0$ in voxels causes spectral shifts of the metabolite peaks, also the lines are broadened when there is variation of $B_0$ inside the voxel of interest, SNR goes down and it might also cause overlap of the metabolite peaks.

Moreover, RF power deposition expressed as SAR (Specific Absorption Rate) increases with $B_0$ which limits the possible number, duration and amplitude of RF pulses that can be used in the sequence and is another limitation at ultrahigh fields like 7T.

On the other hand, SNR generally increases at higher main magnetic field strengths. Pohmann et al for instance found that SNR increases with main magnetic field strength as $B_0^{1.65}$ [19]. Also, there will be greater spectral separation at ultrahigh field as one ppm represents a higher absolute frequency separation at higher field. Moreover, the metabolite peaks at ultrahigh field get simpler. Specifically, the J coupled metabolites' peaks like GABA become simpler at higher main magnetic field strengths because J is constant and independent of main magnetic field strength while spectral resolution increases by increasing the main magnetic field strength. Figure 7 for instance, shows the simplification of J coupled metabolites GABA, Glu and Gln spectra with increasing main magnetic field strength [1]. Also, CSDE increases at higher field strength for the reason explained in the previous section.

T1 (spin-lattice relaxation time) and T2 (spin-spin relaxation time) are main magnetic field (B0) strength dependent and are different for different tissue types. For instance, water T1 increases and water T2 decreases with increasing field strength. There is a similar but less pronounced effect for all metabolites in the brain. T2 relaxation time of macromolecules (MM) are however field independent while their T1 increases with main magnetic field strength [20].

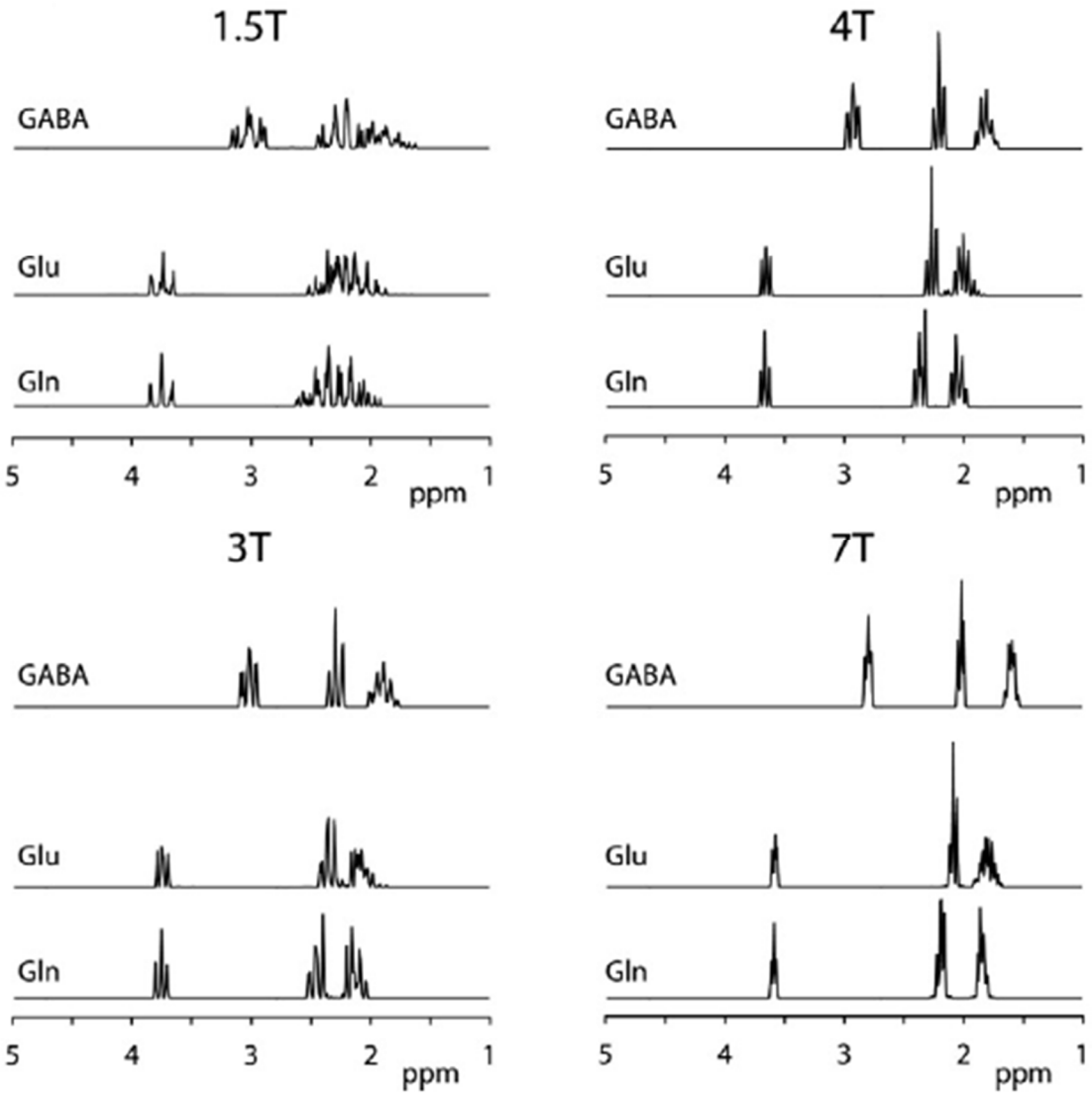
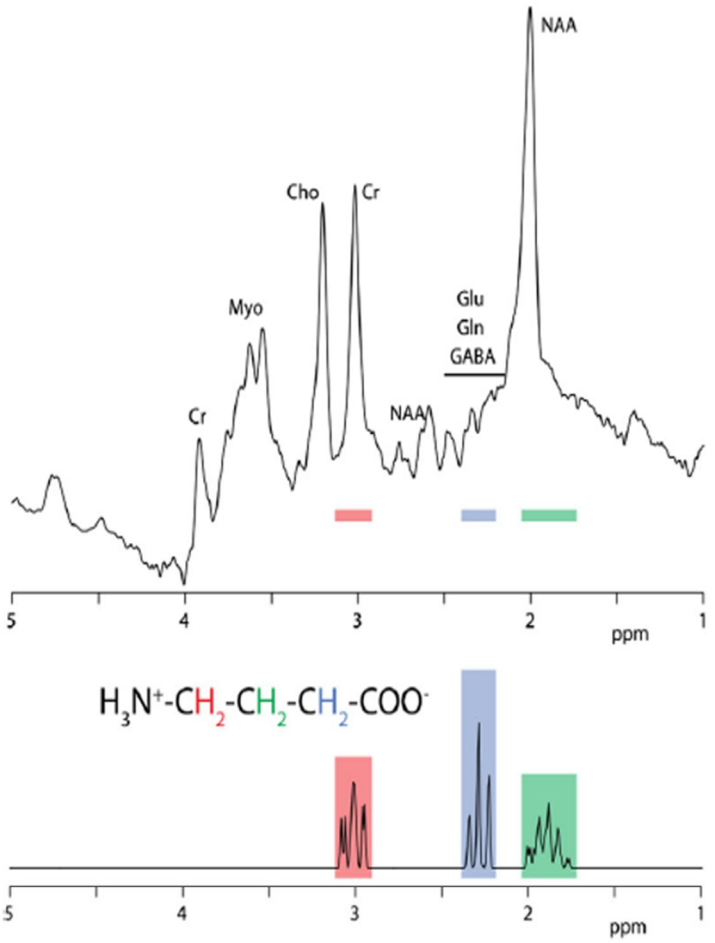



*Figure 7: Pattern of GABA, Glu and Gln spectra at different field strength. The pattern of the J coupled metabolites becomes simpler at higher field strengths, taken with permission from [1].*

## MEGA editing method

Some chemical shifts of some metabolites overlap and therefore it is not possible to distinguish them in a normal spectrum. GABA is among these metabolites. GABA which has a very low concentration in human brain has a multiplet at 3.01 ppm which is overlapped by much more concentrated Creatine that is a singlet. Figure 8 shows the chemical structure of GABA. GABA's proton spectrum consists of three resonances appearing at 1.89 ppm 2.28 ppm and 3.01 ppm which are also shown in Figure 8. There are unequal couplings between the protons which are also given in this figure.




| Spin | Chemical Shift (in ppm) | Couplings (in Hz) to: H2′ | H3 | H3′ | H4 | H4′ |
|---|---|---|---|---|---|---|
| H2 | 2.2840 | −15.938 | 7.678 | 6.980 | 0 | 0 |
| H2′ | 2.2840 | | 6.980 | 7.678 | 0 | 0 |
| H3 | 1.8880 | | | −15.000 | 8.510 | 6.503 |
| H3′ | 1.8880 | | | | 6.503 | 8.510 |
| H4 | 3.0130 | | | | | −14.062 |
| H4′ | 3.0130 | | | | | |

*Figure 8: GABA molecular structure, its three resonances and their position in the proton MR spectrum, and J coupling values, taken with permission from [1,21]*

Among different MRS methods introduced to measure GABA in vivo, MEGA is the most common one [1]. The MEGA editing technique is a method to reveal the J coupled GABA at 3 ppm from the overlapping singlet Creatine. MEGA was introduced as a water suppression

technique [16]. While some water suppression techniques consist of sequence components inserted prior to the localization block like WET [13] or VAPOR [14], MEGA consists of sequence components including RF pulses and spoiler magnetic field gradients that are inserted inside the localization sequence. MEGA's application in addition to water suppression has been shown in measuring GABA [16]. The MEGA editing of GABA is done by inverting 3 ppm GABA's coupling partner at 1.89 ppm. The J coupling between 3 ppm and 1.89 ppm GABA resonances is on average approximately 7.5 Hz. Although, GABA resonance at 3 ppm is a multiplet, at in vivo linewidths at 7T for instance, it appears as a triplet. The two side peaks of GABA at 3 ppm are hence naturally 180 degrees out of phase relative to the central peak at TE = 1/2J = 68 ms. This status of the 3 ppm GABA signal is acquired in a mode named OFF mode where the coupling partner of 3 ppm GABA at 1.89 ppm is not touched. In another mode named ON mode, 1.89 ppm GABA is inverted using two narrow bandwidth editing pulses that are inserted in the localization sequence. Theoretically, one, two or even more frequency selective inversion pulses can be used principally for editing with MEGA technique. Though, two inversion pulses are conventionally used in the implementation of MEGA technique as it was originally introduced for simultaneous water suppression and editing GABA [16]. Refocusing the coupling partner at 1.89 ppm refocuses the 3 ppm GABA. Therefore, in ON mode the side peaks of the 3 ppm GABA will be in phase relative to the central peak. A subtraction of ON and OFF modes thus, eliminates the central peak of GABA as well as the overlapping singlet Creatine, and the two side peaks of GABA will be measured as depicted in figure 9 [22].

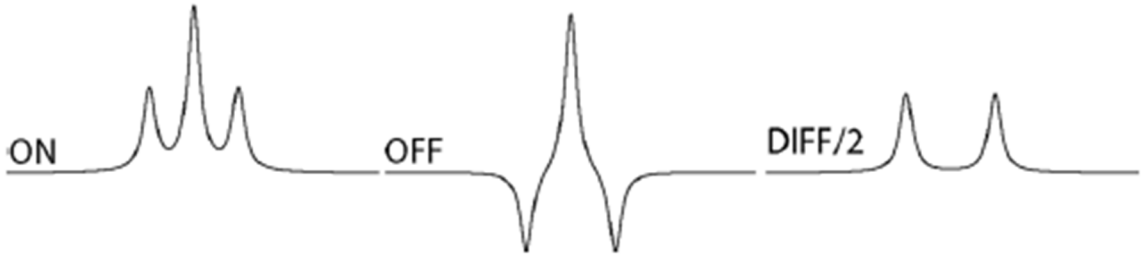



*Figure 9: MEGA editing approach for editing GABA acquires two spectra in two modes named ON and OFF. The difference spectrum reveals the two side peaks of GABA at 3 ppm while the central peak overlapped by Creatine is eliminated, taken with permission from [22].*

The MEGA "suppression and editing" sequence block inserted in a PRESS sequence is shown in Figure 10. The spoiler gradients G1, G2 and G3 are MEGA spoilers that are used for water

suppression. The governing PRESS timing condition along with the two frequency selective inversion pulses for editing GABA are also shown in the sequence diagram.

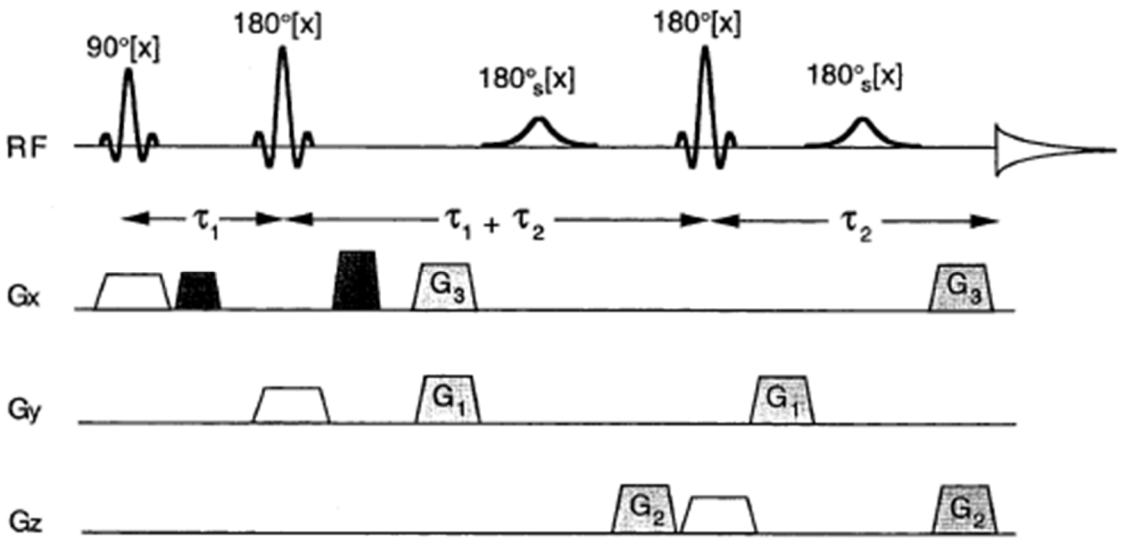



*Figure 10: MEGA-PRESS sequence, taken with permission from [16]*

# Macromolecular contamination of the MEGA edited 3 ppm GABA signal

It is well known that macromolecules can be co-edited with the GABA signal at 3 ppm in MEGA editing method. Behar et al [23] reported presence of coupled MMs at 1.7 ppm and 3 ppm in the human brain *in vivo*. MEGA editing method measures GABA at 3 ppm by inverting its coupling partner at 1.89 ppm. The bandwidth of the editing pulse has therefore an important role in terms of co-editing of the 3 ppm MM. Henry et al [24] proposed an experimental approach to exclude MM from MEGA edited 3 ppm signal. The method is to invert 1.7 ppm in both ON and OFF modes which is done by having inverted 1.9 ppm in one mode and 1.5 ppm in another mode. 1.7 ppm will be then inverted in both modes and therefore subtracted spectrum lacks co-edited 3 ppm MM signal. Another way would be to use very narrow editing pulse which does not invert 1.7 ppm at all. However, as MEGA method for editing GABA is theoretically working best at TE = 68 ms, this echo time brings limitation in terms of using long enough editing pulse to avoid inverting 1.7 ppm.

Although, it is generally thought that MMs possess short T2 relaxation times, there is evidence in the literature for the presence of contaminants presumably MMs in mobile form and with long T2 relaxation time. Choi et al for instance with the aid of double quantum filtering technique found evidence for this [25]. Presence of such contaminants at 3 ppm would impact exact measurement of GABA with J-editing techniques specially at longer TEs.

## Outline of this thesis

In chapter 2 of this thesis, we have compared two commonly used SVS acquisition techniques for measuring GABA at ultrahigh field namely standard sLASER and MEGA-sLASER by implementing them interleaved in a single sequence with the aim of comparing the two methods' efficiency to measure GABA at 7T by acquiring spectra in six different regions of the human brain at 7T. We show in this chapter the key role of 2.28 ppm coedited line of GABA in the LCModel fitting of the MEGA edited spectra which reveals MM free estimation of the 3 ppm edited line.

Among three resonances of GABA in the proton MR spectrum of the human brain, 2.28 ppm peak is not overlapped with any dominant metabolite and therefore is of interest to be measured directly especially at ultrahigh field where this GABA resonance is further away from its neighboring metabolite Glutamate at 2.35 ppm. Ganji et al for instance, found an optimal TE (a long TE = 92 ms) to measure this GABA resonance directly with PRESS sequence at 7T in the human brain [26]. In chapter 3, we present a new version of the sLASER sequence named MASE-sLASER and demonstrate its application to directly measure GABA at TE = 68 ms. Also, the MASE-sLASER sequence employs low power MASE pulses which consequently makes it possible to have them operating at shorter duration than conventional hyperbolic secant pulses. This has brought the possibility of implementing MEGA-sLASER (with MASE) sequence at 7T with TE = 68 ms. Previously, the long duration of conventional adiabatic pulses prevented the implementation of the MEGA-sLASER sequence at 68 ms [9].

Having the two different approaches of MEGA-sLASER and MASE-sLASER to measure two different resonances of GABA at the same TE, in chapter 4, we present MM free estimation of GABA with the aid of LCModel and utilizing the presence of the 2.28 ppm GABA line in the acquired spectra. We also validate these two fundamentally distinct GABA measurement

techniques against each other and get an insight into the relative GABA concentration in pure GM and pure WM by acquiring spectra from predominantly GM and predominantly WM voxels with these two techniques.

In chapter 5, we introduce a new J-editing technique named antiphase editing method and show its application for measuring J coupled metabolites at various TEs; here specifically for measuring GABA. The antiphase editing method's idea emerged from the MEGA editing method. While in MEGA method the two side peaks of GABA at 3 ppm are in phase or out of phase in two modes named ON and OFF, in antiphase editing method we hold the two side peaks of GABA at 3 ppm in antiphase states in two modes. This has made it possible to implement the editing sequence principally at various TEs. The advantage of the antiphase editing method is therefore, that it is not limited to certain TEs while MEGA is. Moreover, as with this editing technique it is possible to implement the sequence at various TEs, this brings the possibility of using long editing pulses in the sequence. Using long enough editing pulses then makes it possible to avoid inverting the MM at 1.7 ppm for instance. So, with this technique and by using very long editing pulses we can investigate the impact of MM on the 3 ppm MEGA edited signal. In addition, use of long editing pulses with narrow bandwidth makes simultaneous editing of 3 ppm and 2.28 ppm lines of GABA feasible with this technique.

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# Chapter 2 - Comparison of sLASER and MEGA-sLASER for GABA measurement using interleaved acquisition at 7T

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## Abstract

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter, is challenging to measure using proton spectroscopy due to its relatively low concentration, J-coupling and overlapping signals from other metabolites. Currently the prevalent methods for detecting GABA at ultrahigh field (> 7 T) are GABA-editing and model fitting of standard single voxel spectra. These two acquisition approaches have their own advantages: the GABA editing approach directly measures the GABA resonance at 3ppm, whereas the fitting approach on the standard spectrum allows the detection of multiple metabolites, and has an SNR advantage over longer TE acquisitons. This study aims to compare these approaches for estimating GABA at 7 T. We use an interleaved sequence of sLASER (TE = 38 ms) and MEGA-sLASER (TE = 80 ms). This simultaneous interleaved acquisition minimizes the differential effect of extraneous factors, and enables an accurate comparison of the two acquisition methods. Spectra were acquired with an 8 ml isotropic voxel at six different brain regions: anterior-cingulate cortex, dorsolateral-prefrontal cortex, motor cortex, occipital cortex, posterior cingulate cortex, and precuneus. Spectral fitting with LCModel quantified the GABA to total Cr (tCr: Creatine + Phosphocreatine) concentration ratio. After correcting $T_2$ relaxation time and tissue volume

variations, GABA/tCr ratios were similar between the two acquisition approaches over six brain regions. GABA editing showed smaller spectral fitting error according to Cramér–Rao lower bound than the sLASER approach for all regions examined. We conclude that both acquisition methods show similar accuracy but the precision of the MEGA-editing approach is higher for GABA measurement. In addition, the 2.28 ppm GABA resonance was found to be important for estimating GABA concentration without macromolecule contamination in the GABA-edited acquisition, when utilizing spectral fitting with LCModel.

# Introduction

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter, is present at about one millimolar concentration in the human brain [1]. GABA plays an important role as a potential biomarker in neurological and neuropsychiatric disorders such as cancer [2], multiple sclerosis [3], Alzheimer's disease [4], epilepsy [5], schizophrenia [6], and autism [7].

Proton Magnetic Resonance Spectroscopy ($^1$H MRS) is currently the only method capable of measuring GABA *in vivo*. However, GABA is a challenging metabolite to measure, due to its low concentration and resonances overlapping with higher concentration signals [8]. Previously, various acquisition strategies for proton MRS have been proposed for measuring GABA, such as J-editing [9] (e.g. MEGA-PRESS (MEsher-GArwood Point RESolved Spectroscopy) [10,11]) and double quantum filters [12].

The MEGA-editing method [8] is the most commonly used approach. A MEGA pulse is used to distinguish the GABA signal at 3 ppm from the overlapping creatine signal, based on the J difference editing approach [10,11]. MEGA-editing combines conventional localization techniques with one or more additional frequency selective editing pulses, which invert the signal at 1.9 ppm. As the GABA resonance at 3 ppm signal is coupled to the 1.9 ppm GABA resonance, the difference between the signals obtained with and without the editing pulses should give an unambiguous GABA signal at 3 ppm. However, the MEGA-editing method also has disadvantages. It theoretically works best at certain echo times (TEs), when the GABA is refocused in anti-phase to the signal obtained without editing pulses (TE = (2n-1) / 2J, where J is 7.35 Hz, n = 1, 2, 3…). Therefore, the TE for GABA editing is largely standardized at 68 ms as the next possible TE beyond 68 ms (204 ms) would imply a significant $T_2$ decay of the signal.

In addition, motion, respiration, and frequency drift induce experimental instabilities that may result in subtraction artifacts [13].

Another extensively used GABA measurement method is to obtain GABA signals from standard spectroscopy spectra on the basis of fitting via LCModel [14,15]. As these data are normally acquired at a shorter TE, the acquired spectrum is superior to the edited spectrum in terms of sensitivity. In addition, not only GABA but also all metabolites are quantified simultaneously.

Given these two leading but different GABA measurement techniques: GABA editing and standard acquisitions, there have been various attempts to assess their relative merits. Terpstra, *et al* [16] compared STEAM (Stimulated Echo Acquisition Mode [17-19], TE = 5 ms) with MEGA-PRESS (TE = 68 ms) at 4 T in a validation study measuring glutathione (GSH). The concentrations of GSH measured with these two methods in that study were similar, suggesting a similar accuracy of the two techniques for those conditions. Sanaie Nezhad, *et al* [20] compared the accuracy and sensitivity of PRESS (Point RESolved Spectroscopy [21,22], TE = 35 ms) and MEGA-PRESS (TE = 130 ms) at 3 T to measure GSH, and found that PRESS is not an accurate and reliable method to measure GSH *in vivo*. They also showed that the spectral fitting of the PRESS spectra cannot reliably quantify the concentration of GSH when the concentration is 4 mM or less. In addition, one conference proceeding by Chen, *et al* [23] reported that the GABA to tCr (creatine + phosphocreatine) ratios from occipital and motor cortices, measured by the MEGA semi-LASER (sLASER: TE = 72ms) and STEAM (TE = 17ms) approaches, are comparable after $T_2$ relaxation correction. However, after macromolecule (MM) correction, GABA editing results were superior to those of the standard spectral acquisitions in terms of both reliability and reproducibility. Nevertheless, the authors still suspected that MM contamination and editing efficiency might have had an influence on GABA quantification.

It is widely known that the 3 ppm signal measured with the GABA-editing technique is typically contaminated by co-edited MM, and this is generally considered to be a methodological limitation of this approach [8,24,25]. Therefore, this measured 3 ppm signal is commonly referred to as GABA+: GABA 'plus' co-edited MM [11,26]. Hitherto, several acquisition methods that

include additional MM elimination strategies have been proposed to minimize the co-edited MM signal at 3 ppm. These include 1) signal nulling that removes unwanted MM signals [27,28] by the pre-inversion method [9]. This technique utilizes the large T1 relaxation time difference between metabolite and MM [29,30]. Sometimes, this technique is used to acquire a metabolite nulled spectrum, which is included as a prior knowledge in quantification in order to quantify metabolites without MM contamination [31,32]. 2) symmetric suppression [33] that applies the editing pulses symmetrically about 1.7 ppm (typically at 1.9 ppm for the editing mode, and at 1.5 ppm for non-editing mode) in order to cancel out the MM signal at 1.7 ppm in the difference spectrum. This technique works on the assumption that the MM signal is symmetrical about 1.7 ppm. 3) Bhagwagar, *et al*. [34] also suggested adding an extra Gaussian model at 3 ppm for LCModel fitting in order to estimate the co-edited 3 ppm MM as a separate signal.

A standard approach to obtain (relative) GABA concentrations from non-edited SVS spectra is to use LCModel [14,15] that fits metabolites together with additional MM, and lipid baselines. Furthermore, the GABA signal is fitted to all three methylene groups of GABA, of which the resonance at 2.28ppm is uncontaminated by MM signal. This should ensure that the GABA estimated in this way does not include MM. We hypothesize that if the MEGA-edited spectrum also contains a visible signal at 2.28ppm then application of LCModel will similarly yield estimates of GABA concentration that are free of MM contamination. We can test this hypothesis by obtaining spectra from the same voxel using both acquisition techniques, to test whether the GABA concentrations obtained are the same. By comparing the spectral fitting quality, we can also assess the relative precision of both methods. We compared relative GABA concentrations at various brain regions of healthy individuals. In order to minimize the effect of external factors, a single interleaved sequence, which acquires spectra of the two measurement methods simultaneously was used. As a result, factors such as motion, shim, and $B_1$ inhomogeneity should be the same for both techniques.

# Methods

## Sequence implementation

The MEGA method can be implemented with conventional SVS acquisition methods. It was initially implemented in a PRESS sequence [10,11,21,22]. However, at ultrahigh field, PRESS loses efficiency because of higher $B_1$ field inhomogeneity. In contrast, the sLASER sequence [35] offers improved performance mainly because of the use of adiabatic refocusing pulses that are insensitive to $B_1$ field inhomogeneity and have a high bandwidth, leading to a small chemical shift displacement error (CSDE) [36].

In this study, the sLASER sequence was used to acquire standard *in-vivo* spectra, and MEGA-editing was performed on the basis of the same sLASER sequence, termed MEGA-sLASER [37]. MEGA-sLASER and sLASER SVS spectra were acquired using a single interleaved sequence shown in Figure 1. It consists of the repeated application of four sub sequence blocks: a MEGA-off sLASER, a MEGA-on sLASER, and two sLASER acquisitions. The repetition time (TR) of each sub sequence block was 4500ms.

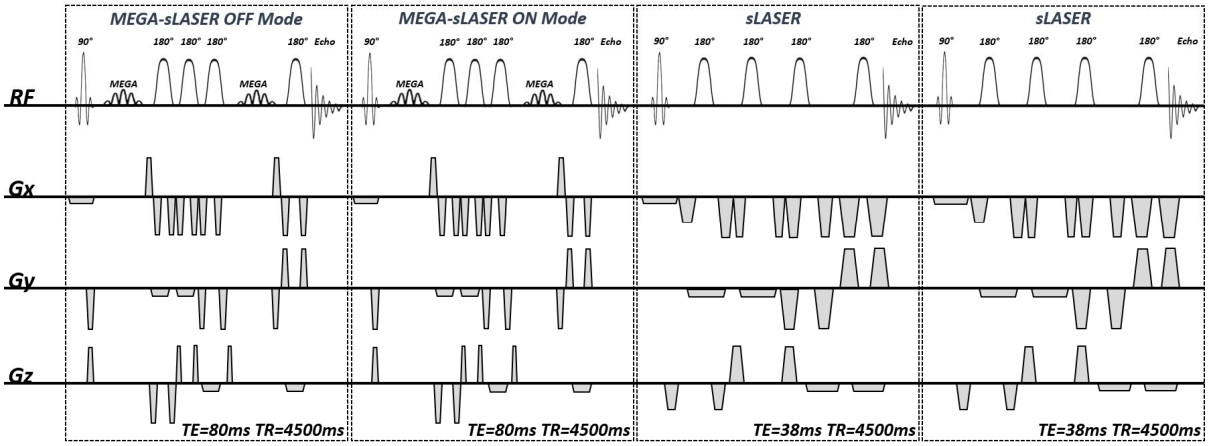


*Figure 1: The pulse sequence diagram of the interleaved MEGA-sLASER (TE = 80 ms) and sLASER (TE = 38ms) sequence implemented in the current study. This sequence is composed of four sub-sequence blocks, which have identical TRs of 4500 ms.*

In the implementation of the sLASER sequence with TE = 38 ms, a Shinnar-Le Roux (SLR) 90º excitation pulse (duration = 3.4 ms and bandwidth = 3.5 kHz) was used for slice selection in

one direction and two pairs of hyperbolic secant 180º refocusing pulses (duration =5 ms and bandwidth = 5.3 kHz) were used for slice selection in the two other directions. The spoiler gradients had an amplitude of 25 mT/m and their duration varied between 0.8 and 2 ms. WET (Water suppression Enhanced through T1 effects) water suppression [38] with four RF pulses was used to suppress the water signal. This has a lower sensitivity to $B_1$ variations compared with the three RF pulses WET water suppression. The water suppression block was placed before the localization block of the sequence and is not depicted in the sequence diagram. TE of 38 ms for this sequence is a slightly conservative choice to ensure elimination of unwanted signals and but comparable to those of other sLASER studies at the same field strength; 24 ms [39], 25 ms [40], 28 ms [6], 32 ms [41] and 36ms [42].

MEGA-sLASER was implemented with TE of 80 ms [43]. Localization components of this sequence were the same as for the sLASER, as described above. A pair of dual-band inversion pulses (duration = 11.52 ms and bandwidth = 133 Hz) was used for both the MEGA-editing and extra water suppression. This editing pulse inverts at 1.9 and 4.7 ppm in on-mode, and at 4.7 and 7.5 ppm in off-mode.

## Data acquisition

12 healthy volunteers (9 male and 3 female subjects, 29.33 ± 4.49 years old) participated with ethical approval from the local ethics committee. The experimental protocol was approved by our institutional review board (IRB). Experiments were performed on a 7 T whole body system (Magnetom, Siemens, Erlangen, Germany) with a 32-channel transmit/receive (TX/RX) bird-cage coil (Nova Medical, Wilmington, MA). First, $T_1$ weighted images were acquired as an anatomical reference using a three-dimensional magnetization-prepared rapid gradient-echo (3D MPRAGE) [44] with the following scan parameters: 256 slices, slice thickness = 1 mm, TR = 2500 ms, TE = 1.35 ms, inversion time (TI) = 1100 ms, flip angle = 6°, field of view = 256 x 256 x 256 $mm^3$, scan time ≈ 5 min. $B_0$ shimming was performed using FASTESTMAP [45]. Single voxel MRS data were collected from 20 x 20 x 20 $mm^3$ voxels positioned at anterior cingulate cortex (AC), dorsolateral prefrontal cortex (DLPFC), motor cortex (MC), occipital cortex (OC), posterior cingulate cortex (PC), and precuneus (PRC) using the interleaved MEGA-sLASER and

sLASER sequence (TR = 4500 ms, TEs = 80, 80, 38 and 38 ms, 32 averages of each sub-sequence, total scan time ≈ 10 min).

## Data processing

The signals from each coil element were combined by the signal weighting method [46] that the manufacturer provides by default. MEGA difference spectra were calculated with the jMRUI software package (Version 6.0, http://www.jmrui.eu) [47,48]. The nominal spectral resolution was increased to 0.27 Hz by zero filling for a more exact adjustment. The MEGA-edited spectra were acquired by subtracting MEGA-on and MEGA-off spectra after alignment with the 3ppm peak by the horizontal shift along the frequency axis, to avoid subtraction artifacts [13] caused by frequency drift. After subtraction, 1 Hz line broadening was applied to remove the zero-filling induced high-frequency noise. The same level of zero-filling and line broadening were applied to the GABA editing and the standard acquisition spectra.

## Data analysis

Spectral quality was evaluated on the basis of the SNR, and was measured using the peak amplitude of the total NAA peak at 2 ppm and the standard deviation of the white noise area in the 7 to 9 ppm range, for both GABA-editing and standard acquisition spectra.

LCModel software (Version 6.3-1L, Stephen Provencher, Ontario, Canada) [14,15] was used to estimate metabolite concentrations. The Cramér–Rao lower bound (CRLB), was taken as giving the error in the metabolite quantification (expressed in %SD) [14]. For the basis set for the LCModel analysis, parametric spectral models of alanine (Ala), aspartate (Asp), ascorbate (Asc), glycerophosphocholine (GPC), choline (Cho), phosphocholine (PCh), creatine (Cr), phosphocreatine (PCr), GABA, glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), GSH, myo-inositol (mI), lactate (Lac), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), scyllo-inositol (Scyllo) and taurine (Tau) were simulated using the NMRSIM module included in TOPSPIN suite (Version 3.6, Bruker, Rheinstetten, Germany). For each of the two acquisition sequences the basis set intensities were generated using TOPSPIN with identical parameters (e.g. RF pulse profile, resonance frequency and acquisition bandwidth) to those used for the in-vivo acquisition. Chemical shift and J-coupling values for each metabolite were taken from [49].

For the sLASER data, the basis set for the LCModel analysis consisted of all twenty-one simulated metabolites. For the GABA editing method, six edited metabolites were modelled: GABA, Glu, Gln, NAA, NAAG, and GSH were included in the basis set. Each edited spectral model was created by subtracting a simulated MEGA-off spectrum from a simulated MEGA-on spectrum. As LCModel performs a phase correction during spectral fitting, additional phase correction steps were not applied. For MM and lipid signals, the non-parametric basis sets that LCModel provides by default were used [50]. However, one singlet peak with Lorentzian lineshape was also included in the GABA-editing basis set model the MM signal at 3 ppm, and thus avoid over-estimation of GABA due to the co-edited MM [34].

We report GABA concentrations as a ratio relative to tCr across the brain regions. We used the tCr signal in the MEGA-off spectrum as an internal reference for the MEGA-editing approach. We accepted metabolite concentrations only with CRLB values of under 50% for the statistical analysis in accordance with the recommendation of LCModel [50]. As the data were acquired at different TEs, the signal concentration differences were also compensated [51] for $T_2$. We assumed similar $T_2$ relaxation times in GM and WM for each metabolite, and used values taken from the literature of: $T_{2\ tCr}$ = 121 ms and $T_{2\ GABA}$ = 63 ms [52] at 7 T. In addition to the $T_2$ relaxation time, it is widely known from previous *ex-vivo* [53] and *in-vivo* [54-56] studies that GABA is very unequally distributed between gray matter (GM) and white matter (WM). Therefore, tissue volume variation inside the spectroscopy voxel should be considered for the GABA quantification, as each volume will have relatively different tissue volume fractions [57]. T1 weighted images of each spectroscopy voxel of interest (VOI), which were also used for spectroscopy voxel placement, were segmented using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK) unified segmentation routines to determine the relative proportions of GM, WM and cerebrospinal fluid (CSF) within the spectroscopy voxel. We calculated a volume fraction for each tissue component. GABA and Cr signal concentrations for each spectroscopy voxel were weighted by their tissue volume fractions on the assumption that GABA is concentrated 89.9%: 10.1% [53] and tCr is 57.1%: 42.9% [58] in GM:WM, respectively. The GABA to tCr concentrations of each voxel were weighted by

$$\left(\frac{S_{GABA}}{S_{tCr}}\right)_{CORR} = \left(\frac{S_{GABA}}{S_{tCr}}\right)_{MEAS} \left(\frac{f_{GM}*D_{GMtCr}+f_{WM}*D_{WMtCr}+f_{CSF}*D_{CSFtCr}}{f_{GM}*D_{GMGABA}+f_{WM}*D_{WMGABA}+f_{CSF}*D_{CSFGABA}}\right) \quad (1)$$

where $S_{CORR}$ and $S_{MEAS}$ are the ratio of the corrected and measured metabolite signals, $f_{GM}$, $f_{WM}$ and $f_{CSF}$ are tissue volume fractions, and $D_{GM}$, $D_{WM}$ and $D_{CSF}$ are the fractional metabolite distributions in the GM WM and CSF, respectively. We assumed that CSF was not expected to contain significant quantities of metabolites and hence set $D_{CSF}$ to zero [59].

A paired-sample t-test was performed to compare both the concentration ratio and the CRLB between the two acquisition approaches. In addition, regional GABA concentrations were also compared with a one-way ANOVA. All statistical tests were performed using SPSS (Ver. 22, IBM, NY). The null hypothesis was that there is no difference between the results obtained with the two acquisition methods and regions. A p-value less than 0.05 was considered statistically significant.

Potential MM contributions in the edited 3ppm peak of the MEGA approach were also approximated by amplitude ratios between the fitted GABA line by LCModel and the acquired GABA+ peak at 3 ppm.

# Results

Water linewidths obtained after performing 2 - 3 iterations with the FASTESTMAP sequence were below 14 Hz for all voxels. Figure 2A depicts locations of VOIs on the GM (gray) and WM (white) maps after T1 weighted image segmentation. Figure 2B shows examples of fitting GABA with LCModel using sLASER (top) and the MEGA-sLASER (bottom) for all six regions. Table1 summarizes mean metabolic concentrations with their CRLB values at six different brain regions for the two different acquisition approaches. Measured spectral SNRs are also summarized in Table 1.

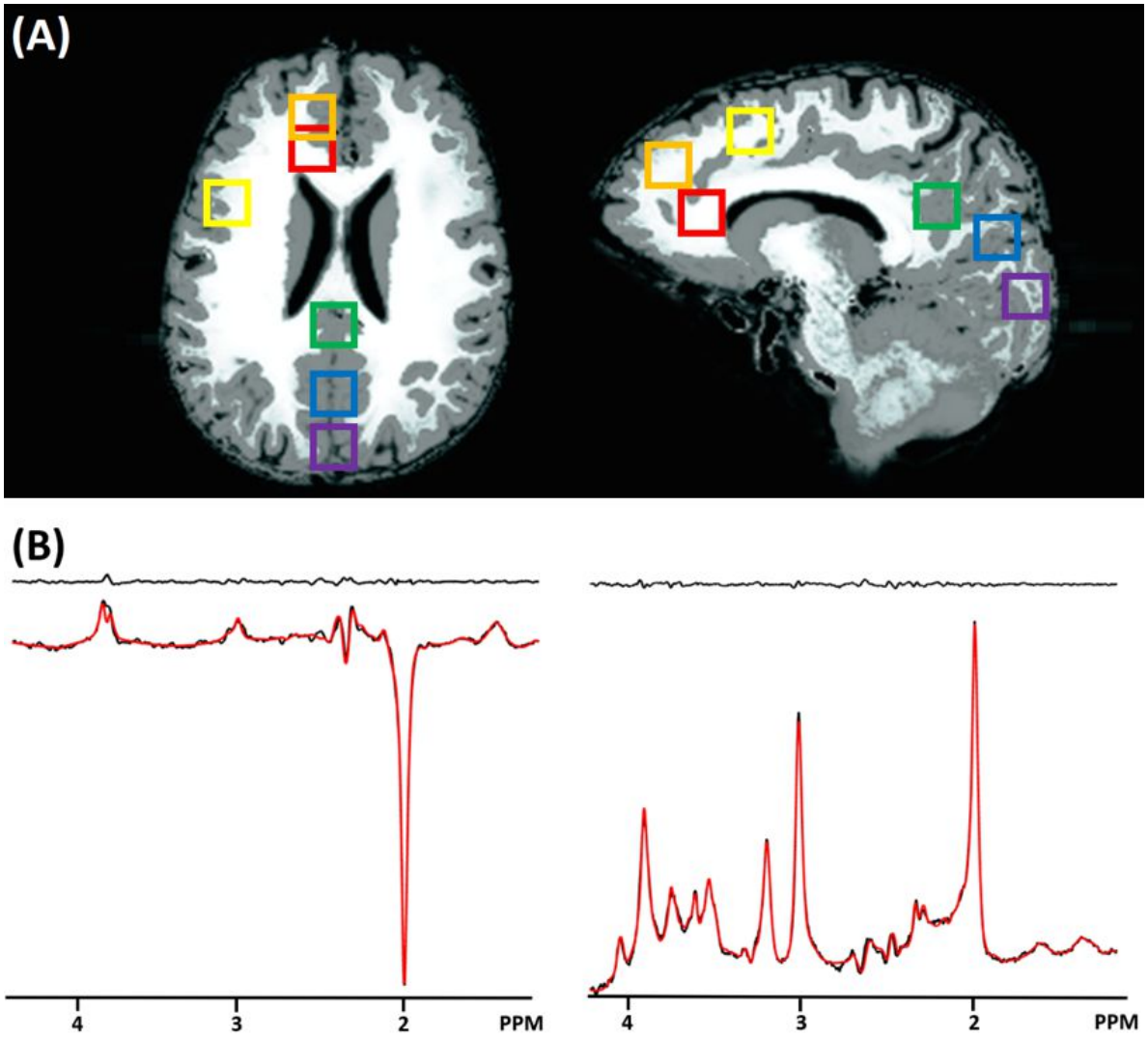


*Figure 2: (A) six spectroscopy voxel positions : anterior cingulate cortex (AC: red), dorsolateral prefrontal cortex (DLPFC: orange), motor cortex (MC: yellow), occipital cortex (OC: purple), posterior cingulate cortex (PC: green), and precuneus (PRC: blue). on the GM (gray) and WM (white) map. (B) example results of the LCModel analysis for the MEGA-sLASER (left) and the sLASER (right) that were acquired with the interleaved acquisition from a single voxel (PRC). The fitted*

| | SNR | | Concentration GABA/tCr | | CRLB(%) GABA | | tCr | |
|---|---|---|---|---|---|---|---|---|
| Technique | sLASER | MEGA-sLASER | sLASER | MEGA-sLASER | sLASER | MEGA-sLASER | sLASER | MEGA-sLASER |
| TE (ms) | 38 | 80 | 38 | 80 | 38 | 80 | 38 | 80 |
| AC | *511.80 ± 90.25* | *325.17 ± 62.40* | *0.128 ± 0.068* | *0.120 ± 0.037* | *41.20 ± 8.42* | *22.80 ± 6.27* | *1.00 ± 0.00* | *2.20 ± 0.75* |
| DLPFC | *435.37 ± 146.20* | *388.87 ± 93.90* | *0.224 ± 0.085* | *0.147 ± 0.023* | *35.67 ± 7.07* | *26.00 ± 6.68* | *1.33 ± 0.47* | *1.13 ± 0.47* |
| MC | *540.78 ± 155.41* | *294.18 ± 57.73* | *0.117 ± 0.017* | *0.173 ± 0.076* | *36.40 ± 4.13* | *28.00 ± 1.72* | *1.20 ± 0.40* | *1.15 ± 0.40* |
| OCC | *417.24 ± 37.00* | *410.63 ± 126.73* | *0.260 ± 0.050* | *0.312 ± 0.078* | *28.86 ± 7.18* | *28.14 ± 9.16* | *1.28 ± 0.45* | *1.14 ± 0.35* |
| PC | *441.11 ± 102.62* | *277.74 ± 78.50* | *0.246 ± 0.087* | *0.262 ±0.056* | *31.86 ± 4.32* | *27.16±13.74* | *1.14 ± 0.35* | *1.25 ± 0.43* |
| PRC | *487.66 ± 143.81* | *314.86 ± 102.78* | *0.247 ± 0.046* | *0.261 ± 0.047* | *31.00 ± 6.52* | *26.28 ± 6.13* | *1.14 ± 0.35* | *1.14 ± 0.35* |

*Table 1: Averaged LCModel results from six brain regions by the sLASER and the MEGA-sLASER approaches. LCModel estimated relative concentrations to tCr with CRLB of detected metabolites for the sLASER and MEGA-sLASER. These metabolic concentrations are directly measured values by LCModel before correcting for tissue composition and T2-relaxation. The measured value was expressed as mean ± SD*

Figure 3 shows the correlation between GABA/tCr and GM volume fraction in the spectroscopy voxel estimated with the two different acquisitions after $T_2$ correction between sLASER (TE = 38 ms) and MEGA-sLASER (TE = 80 ms). We found a linear correlation between the GABA/tCr concentration ratio and GM tissue distribution (linear regression line: $y_{TE=38\,sLASER} = 29.27x + 4.68$ and $y_{TE=80\,MEGA-sLASER} = 33.18x + 2.54$, %). In addition, these regression lines were almost identical for the two methods. This result shows a large dependence of measured GABA concentration on GM volume fraction, independent of the two acquisition methods. On the assumption that CSF does not contain significant quantities of metabolites [59], estimated percentage GABA distributions in the GM and WM using the linear regression lines were (GM%: WM%) 86%: 14% for sLASER and and 93%: 7% for MEGA-sLASER, very much in line with the literature values for the relative distribution between GM and WM [53].

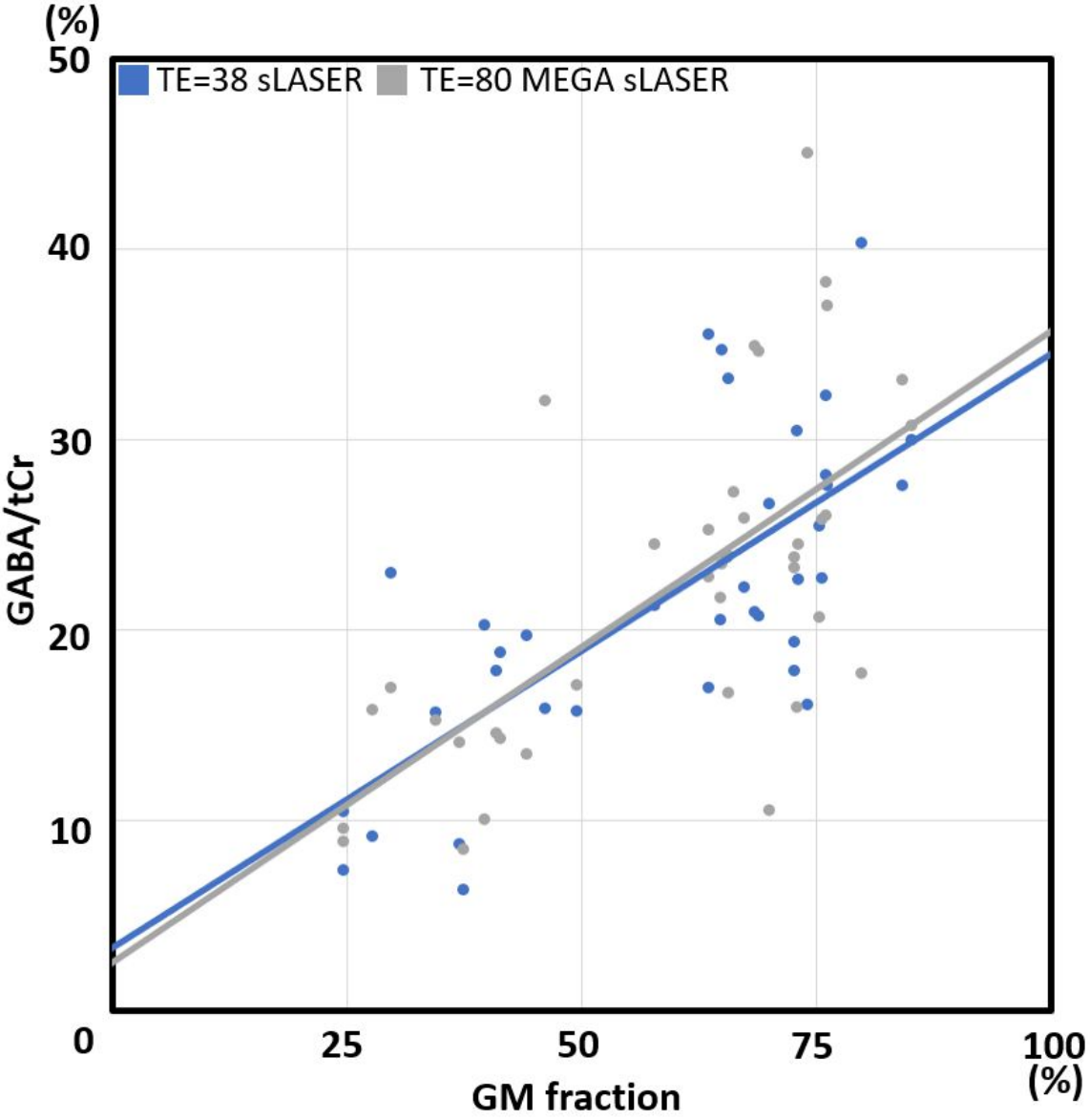


*Figure 3: Correlation between GABA/tCr and GM volume fraction in the spectroscopy voxel measured by two different approaches (blue: TE = 38 sLASER, gray: TE = 80 MEGA-sLASER) across all examined voxels after the $T_2$ correction. GABA concentration was in direct proportional to the GM tissue fraction. Linear regression lines of these two methods are almost identical. Equations of the linear regression lines were* $\boldsymbol{y_{TE=38\ sLASER} = 29.27x + 4.68}$ *and* $\boldsymbol{y_{TE=80\ MEGA-sLASER} = 33.18x + 2.54}$*, unit(%).*

In order to exclude the effect of tissue composition variations on the GABA quantification, we also corrected for differences tissue volume fractions using equation (1). After compensating the $T_2$ relaxation time of GABA and NAA, and the GM/WM fraction variation for each voxel, we found similar GABA to tCr ratios acquired with the two methods for each voxel (Figure 4A).

Error bars indicate 95 % confidence intervals. From the fact that p-values between the two acquisition approaches were above 0.05 for all voxels, the null hypothesis that both techniques yield the same relative concentrations is satisfied. In addition, regional GABA concentrations showed no statistically significant differences between group means as determined by a one-way ANOVA ($F(5,36) = 0.092$, $p = 0.996$) for the sLASER method, and ($F(5,36) = 0.585$, $p = 0.711$) for the GABA editing method. Even though regional GABA concentrations are not statistically different, OC showed the highest mean GABA/tCr ratio as compared with the other five regions, where GABA/tCr ratios were similar.

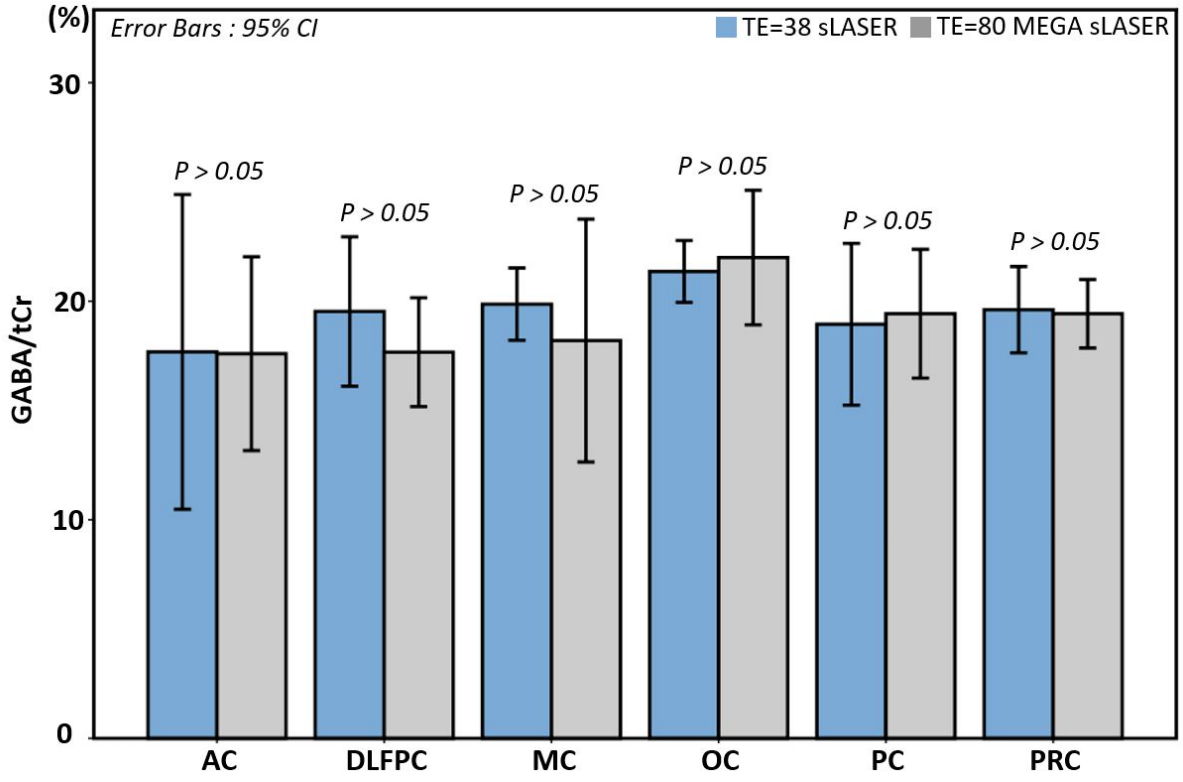


*Figure 4: GABA/tCr comparisons after considering both the GM and WM volume weighting and the $T_2$ relaxation times of GABA and NAA. $T_2$ correction factor for TE=80 MEGA-sLASER was 1.488 for GABA/tCr. After applying this factor, we found no statistically significant difference in GABA/tCr ratio between the two methods. P-values were above 0.05 for all regions. Error bars indicate 95% confidence intervals.*

Figures 5 shows CRLB comparisons of GABA (A) and tCr (B), estimated by the two different acquisition strategies. For both metabolites, the MEGA-editing approach showed slightly better spectral fitting quality than that of the standard acquisition approach according to CRLB for all regions examined. Error bars also indicate 95% confidence intervals.

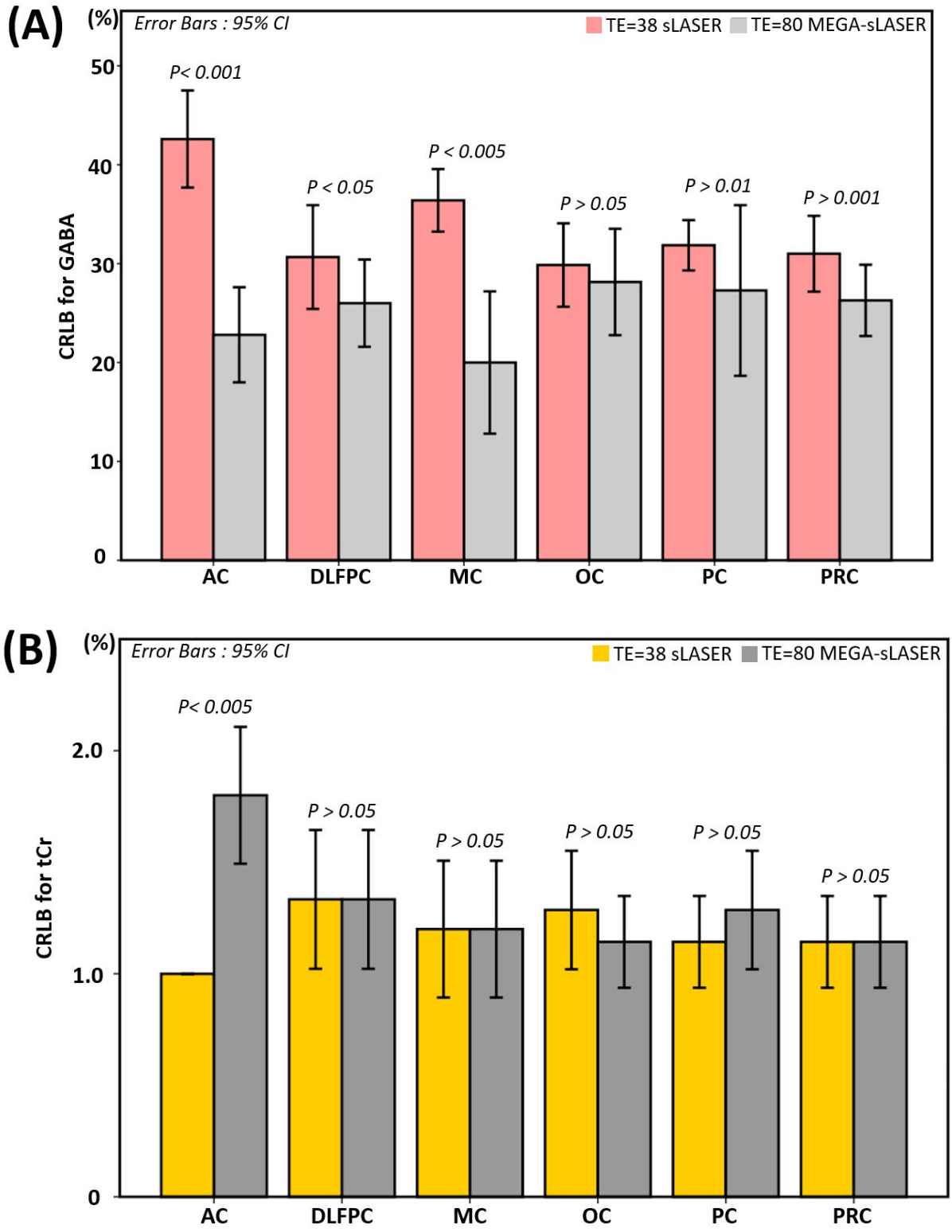


*Figure 5: CRLB comparison of (a) GABA and (b) tCr between sLASER and MEGA-sLASER for the six brain regions examined. Error bars indicate 95% confidence intervals, in accordance with table 1.*

One example of the GABA edited spectrum (gray) with metabolite fitting lines: GABA (red), NAAG (green), NAA (purple), Gln (yellow) and Glu (blue), which were estimated from LCModel is shown in Figure 6. GABA estimation is clearly to a large degree determined by the fit to the 2.28 peak. Pure GABA accounts for 47.21 ± 17.04 percent of the total 3ppm signal, calculated as the ratio of the LCModel GABA fitted line at 3ppm to the total measured 3 ppm peak averaged over all subjects.

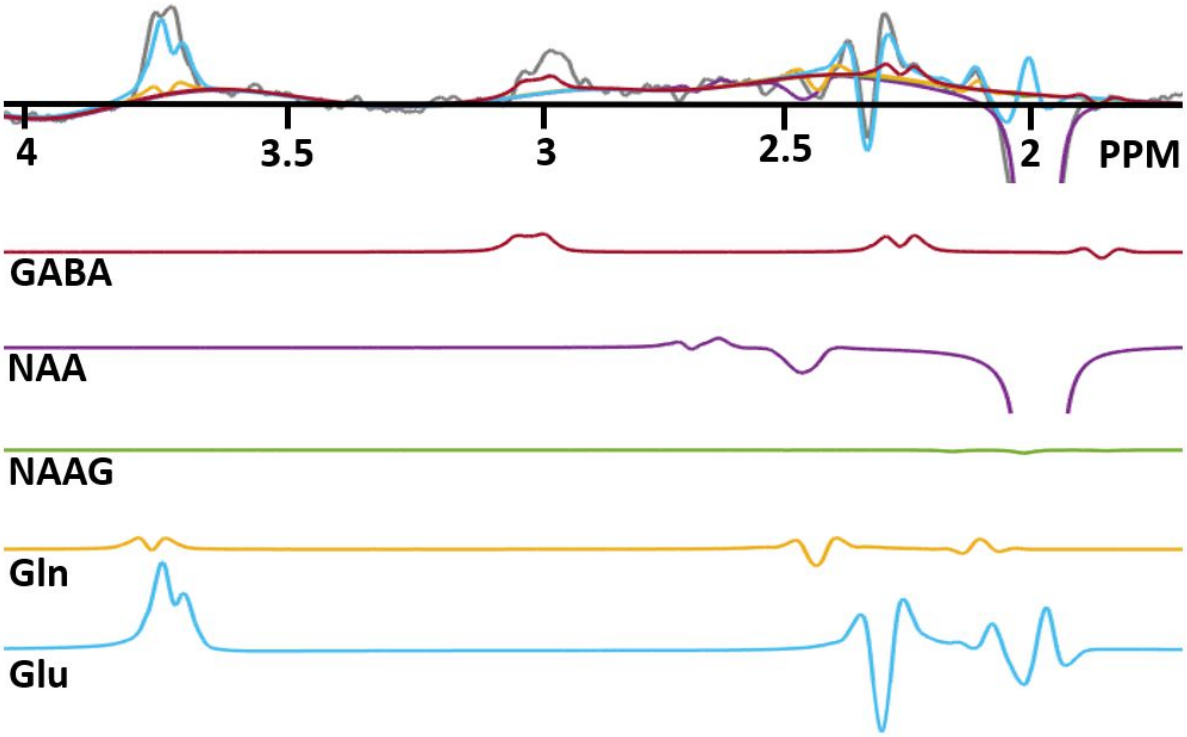


*Figure 6: one example of GABA edited spectrum (gray) with metabolite fitting lines: GABA (red), NAAG (green), NAA (purple), Gln (yellow) and Glu (blue), which were estimated from LCModel. Directly observable GABA-$^2CH_2$ resonance at 2.28 ppm plays an important role in the GABA quantification with spectral fitting. GABA estimation is limited by the 2.28 peak, which is the true GABA. It prevents over-estimating GABA by co-edited macromolecule. Note that the bottom part of the NAA signal is truncated in this figure.*

# Discussion

The interleaved sequence, which we implemented in this study, provides simultaneous MEGA-editing and standard acquisitions in a single sequence. By acquiring concurrently, the interleaved acquisition balances any instability across the two techniques. Therefore, we could avoid potential problems of registration, motion, $B_1$-inhomogeneity or different shim quality that may have occurred if we would have acquired sequentially. After applying the $T_2$

correction factor, which compensates the different TEs, the GABA/tCr ratios for the two acquisition techniques did not differ significantly.

## Regional GABA Concentration

As we mentioned in the Introduction, it is widely known that GABA is mostly concentrated in GM (approximately 90% in GM and 10% in WM). Not only spectroscopy studies [54-56] but also an *ex-vivo* study [53] obtained similar GABA distribution ratios between GM and WM. Chen, *et al* [60] reported that GABA concentration linearly increased with GM volume fraction for one of the brain regions we also studied (AC). In our result, as we can see in figure 3, GABA concentrations were largely driven by the GM tissue fraction. In addition, estimated GABA distribution ratios in the GM and WM by the linear regression lines are in close agreement with the literature value. This result confirms that GABA is heavily concentrated in GM, and highlights the importance of the tissue volume correction for GABA quantification [57].

In addition to the current study, other studies, which investigated regional GABA concentration differences while accounting for tissue volume variations, found differences in the regional GABA level. However, there is some inconsistency in the literature. For instance, Ganji, *et al* [56] reported higher concentrations of GABA in the frontal lobe than in the OC at using PRESS (TE = 92 ms) at 7 T. Öngür, *et al* [61] found higher GABA/Cr ratio in a frontal region (AC) than in an occipital region (parieto-occipital cortex) measured with the MEGA-editing method at 4 T. On the other hand, Bhagwagar, *et al* [62] found higher GABA/Cr ratio measured with MEGA-editing in the occipital region (OC) than that in AC. Durst, *et al* [63] also reported higher GABA in OC than in frontal lobe using 3 T MEGA-PRESS. Van der Veen, *et al* [64] similarly found higher GABA concentration in the OC than that of the anterior area (MPFC) with the MEGA-editing method comparing two voxels in frontal and occipital lobes. Van der Veen, *et al* [64] selected voxels that had the same GM and WM tissue volume to exclude tissue volume effects. They proposed therefore that the difference should be attributed to factors other than GM volume. The trend in our data is in accordance with that of the latter studies [62-64].

Recent molecular Imaging studies, which quantified $GABA_A$ receptor using positron emission tomography (PET) with $^{11}C$ flumazenil [65,66], and single-photon emission computed tomography (SPECT) with $^{123}I$-iomazenil [67] have visualized $GABA_A$ receptor densities in the human brain.

These reports have shown not only that the $GABA_A$ receptor is more heavily concentrated in GM, but also its density in posterior brain areas is higher than that of anterior areas. This supports our finding that GABA concentration in OC showed the highest level.

## MM contamination

We found no statistically significant difference for the quantified GABA concentrations with LCModel between two acquisition techniques. Our finding could potentially be explained by one of two possibilities: The LCModel fitting result of the standard spectrum also contains MM contamination, or LCModel efficiently quantifies the GABA signal from the GABA edited spectrum without the interference of the co-edited MM.

LCModel estimates MM and lipid signals at each fixed chemical shift location with non-parametric spectral models including a smoothing B-spline as a baseline [14]. Because these MM and lipid signals are not only measured values but also part of the formulaic model, these quantification approaches still risk over-, or under-estimating metabolic concentrations in the co-edited signal. It is quite challenging to determine from our LCModel quantification results of sLASER whether the estimation of GABA is contaminated by MM. However, we can assume the presence of MM contamination in the MEGA-sLASER spectra. In contrast to MM and lipid signals, metabolic signal quantification uses parametric spectral models, which are included in a basis set. LCModel finds metabolic concentrations by changing the amplitudes of simulated metabolic models with their lineshapes unchanged. Therefore, all resonances of GABA also play an important role in solving the optimization problem. In our spectral fitting results, we found that the directly discernible 2.28ppm GABA signal was essential for distinguishing the GABA signal from GABA+ (See Figure 6). GABA estimation was largely determined by the size of GABA-$^2CH_2$ resonance at 2.28 ppm. Because the 2.28ppm region is not related to the co-edited MM, the visible GABA resonance at 2.28ppm represents an uncontaminated GABA signal. This result suggests that GABA quantification with spectral fitting is able to separate pure GABA from GABA+ provided that the 2.28ppm GABA resonance is also recorded. Previous studies by Ganji, *et al* [56] and Choi, *et al* [68] also highlighted the significance of this GABA-$^2CH_2$ resonance in terms of spectral fitting procedure.

LCModel provides a GABA-editing exclusive control file called 'mega-press-3' [50]. This setting excludes quantification of the baseline and MM signals assuming baseline and MM signals are identical between MEGA-on and -off spectra, and that they hence cancel in the subtraction. Since this setting mainly attempts to estimate the 3ppm peak as GABA+, this necessarily overestimates the GABA concentration as co-edited MM will not be eliminated. Therefore, we did not use this approach.

We corrected for $T_2$ relaxation using the T2-value of 63 ms taken from the study by Intrapiromkul, *et al* [52]. After correction the values obtained were similar between the two methods (See Figure 3 and Figure 4a). This consistency strongly supports the correctness of the T2-value used, and also that the analyses have yielded results without MM contamination. Our estimate that MM constitutes approximately 53% of the signal at 3ppm is at the upper limit of, but not inconsistent with previous literature values 44% [9], 41 - 49% [69], and 52-57% [70]

## Spectral fitting quality

We found that CRLBs of estimated GABA for the MEGA-editing method are slightly lower than those for standard acquisition method for all six regions (See Figure 5A). Compared with the standard acquisition, the GABA editing method provides a much simpler spectral pattern with only six metabolic signals present, that are relatively well separated. This explains why the spectral fitting accuracy of the MEGA editing results for estimating GABA is superior to that of the sLASER result, despite the editing method eliminating also number of lines from the GABA spectrum. MEGA-sLASER and sLASER showed similar CRLB values for tCr (Figure 5B) which is unsurprising given that the tCr concentration of the MEGA-sLASER was estimated from the MEGA-off spectra using a standard acquisition. The reasonably good CRLB values of tCR for both approaches confirms that spectral quality and spectral fitting quality with LCModel were good enough for accurate quantification.

# Conclusion

This study compared GABA concentration for six different brain regions with the standard spectral acquisition approach and the GABA editing approach using a single interleaved sequence acquisition. The simultaneous acquisition of sLASER and MEGA-sLASER minimized

differences in confounds which could have occurred in individual acquisitions. Our finding confirms that GABA is heavily concentrated in GM. Therefore, regional GM volume is an important contributor to measured GABA concentration variation. In addition, the ability to reliably measure the GABA resonance at 2.28ppm would appear to lead to accurate estimates of GABA concentration without MM contamination. A similar concentration of GABA signal measured with the two methods for each voxel was obtained after correction for $T_2$ and tissue content, showing that the two acquisition strategies are both reliable and have a similar accuracy to measure GABA. However, The GABA-editing approach was superior in terms of precision, as assessed by lower CRLB values.

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# Chapter 3 - MASE-sLASER

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## Abstract

$B_1$ inhomogeneity and chemical shift displacement error (CSDE) increase with the main magnetic field strength and are therefore deleterious for Magnetic Resonance Spectroscopy (MRS) at ultrahigh field. A solution is to use adiabatic pulses which operate over a broad range of $B_1$ and thus, are insensitive to $B_1$ inhomogeneity. Moreover, adiabatic pulses usually have relatively higher bandwidth which makes CSDE low to negligible. Use of exclusively adiabatic pulses for single voxel spectroscopy (SVS) typically brings the disadvantage of TE being long but the advantage of CSDE being low and matched. Herein, we took advantage of short duration and low power, matched phase adiabatic spin echo (MASE) pulses to implement a matched CSDE sLASER sequence capable of attaining short TEs, while CSDE is matched and still comparatively low. We also demonstrate here the feasibility of direct measurement of GABA resonance at 2.28 ppm well separated from the neighboring Glutamate resonance at 7T using the implemented MASE-sLASER sequence at TEs of 68 and 136 ms. The shorter duration of MASE pulses also made it possible to implement a MEGA-sLASER [with MASE] sequence with TE = 68 ms for editing GABA at 7T: results for which are also shown.

## Introduction

Magnetic resonance spectroscopy (MRS) at ultrahigh magnetic field strengths benefits from the advantages of increased signal-to-noise ratio (SNR) as well as greater spectral separation between metabolite peaks. However, both $B_1$-field inhomogeneity and chemical shift displacement error (CSDE) increase at ultrahigh field [1,2]. These problems can be ameliorated

by utilization of adiabatic pulses which operate over a broad range of $B_1$-field strengths, and have higher spectral bandwidth [3,4,5,6].

Examples are the SADLOVE sequence [7] and LASER sequence [4] which use only adiabatic RF pulses. This makes these sequences largely immune to $B_1$-field inhomogeneity which is an advantage at ultrahigh field. On the other hand, the use of seven RF pulses in SADLOVE and LASER sequences makes them so long that short TE single voxel spectroscopy (SVS) acquisition is currently not possible with them at ultrahigh field. As an alternative, the semi localized adiabatic selective refocusing (sLASER) sequence [8,9] was introduced which has the advantage of relatively shorter TE while having the residual disadvantage of sensitivity to $B_1$-field inhomogeneity along one axis because of the use of a non-adiabatic excitation pulse.
In addition to $B_1$-field inhomogeneity, another problem in SVS at ultrahigh field is CSDE [9]. This artifact increases linearly with field strength. In order to deal with this issue, high bandwidth RF pulses are needed. This requirement limits for example the use of sinc-like (refocusing) pulses for PRESS spectroscopy at ultrahigh field because of the need for very high peak voltages to achieve the necessary bandwidth. Here also, use of adiabatic pulses which have high bandwidth is a potential solution. For instance, the sLASER sequence benefits from lower CSDE in two refocusing directions because of the use of high bandwidth adiabatic refocusing pulses [9]. However, the sequence typically still has a larger CSDE in the excitation direction because of the use of a non-adiabatic RF pulse with a lower bandwidth than adiabatic pulses.

MEGA-sLASER [10] is currently the most common MRS approach to measure GABA in vivo. Theoretically, the most efficient TE to measure GABA is 68 ms [11] where the two side peaks of GABA are refocused (ON mode) and inverted (OFF mode) [12]. The requirement for relatively long conventional adiabatic pulses and long editing pulses in the implementation of the MEGA method at ultrahigh field limits the minimum achievable echo time to longer than 68 ms. For instance, the MEGA-sLASER sequence was first introduced and implemented as an efficient method to measure GABA at 7T with TE = 74 ms [10].

The proton magnetic resonance spectrum of GABA includes three coupled peaks appearing at 1.89, 2.28 and 3.01 ppm in vivo [12]. GABA at 3.01 ppm overlaps with the Creatine singlet peak and therefore J difference editing methods like MEGA-sLASER are currently the most common

way to measure this GABA signal. GABA at 2.28 ppm does not entirely overlap with any prominent neighboring metabolite peaks, however it is necessary to distinguish it from the proximal glutamate peak at 2.35 ppm. Higher spectral resolution at ultrahigh field therefore provides the possibility of separation of these two neighboring signals in the acquired spectrum. Ganji et al for instance [13], showed an optimum PRESS at TE = 92 ms at 7T to have these two neighboring signals best separated from each other.

Recently, matched-phase adiabatic spin echo (MASE) pulse pairs have been introduced and their application shown in diffusion weighted imaging [14]. MASE includes a non-adiabatic matched phase SLR 90 pulse and an adiabatic SLR 180° pulse where the SLR 90 pulse is used to compensate for the nonlinear phase of the adiabatic SLR 180° pulse across the slice, creating a spin echo without the need for a pair of adiabatic refocusing pulses [14]. This characteristic of MASE can be beneficial at ultrahigh field by reducing possible TE in comparison to a full LASER sequence. A feature of MASE pulses is that they require less power than hyperbolic secant pulses. Consequently, they can operate at shorter duration while having an acceptable bandwidth. These features enable the implementation of the MEGA editing method with TE of 68 ms for editing GABA at ultrahigh field. Whereas previous editing implementations at 7T using sLASER like techniques have been forced to use TEs higher than 68 ms (74 ms [10,15]).

Hence, our aim in this study is to implement a sequence for short TE full intensity SVS with matched and low CSDE in all three directions for general use (MASE-sLASER) and for editing GABA with TE = 68 ms (MEGA-sLASER (with MASE)) at 7T. We also examine the feasibility of direct measurement of the GABA resonance at 2.28 ppm using the MASE-sLASER sequence.

# Methods

## Implementation of the sequences

### MASE-sLASER

A short TE = 27 ms matched CSDE sLASER sequence (MASE-sLASER) (Figure 1) was implemented using MASE for slice selection in one direction and two pairs of MASE SLR refocusing pulses in the two other directions. The slice selection gradient strength of the

excitation pulse of MASE is matched with that of the refocusing pulse(s) of MASE. This gives matched slice selection gradient strengths and CSDE in all three directions.

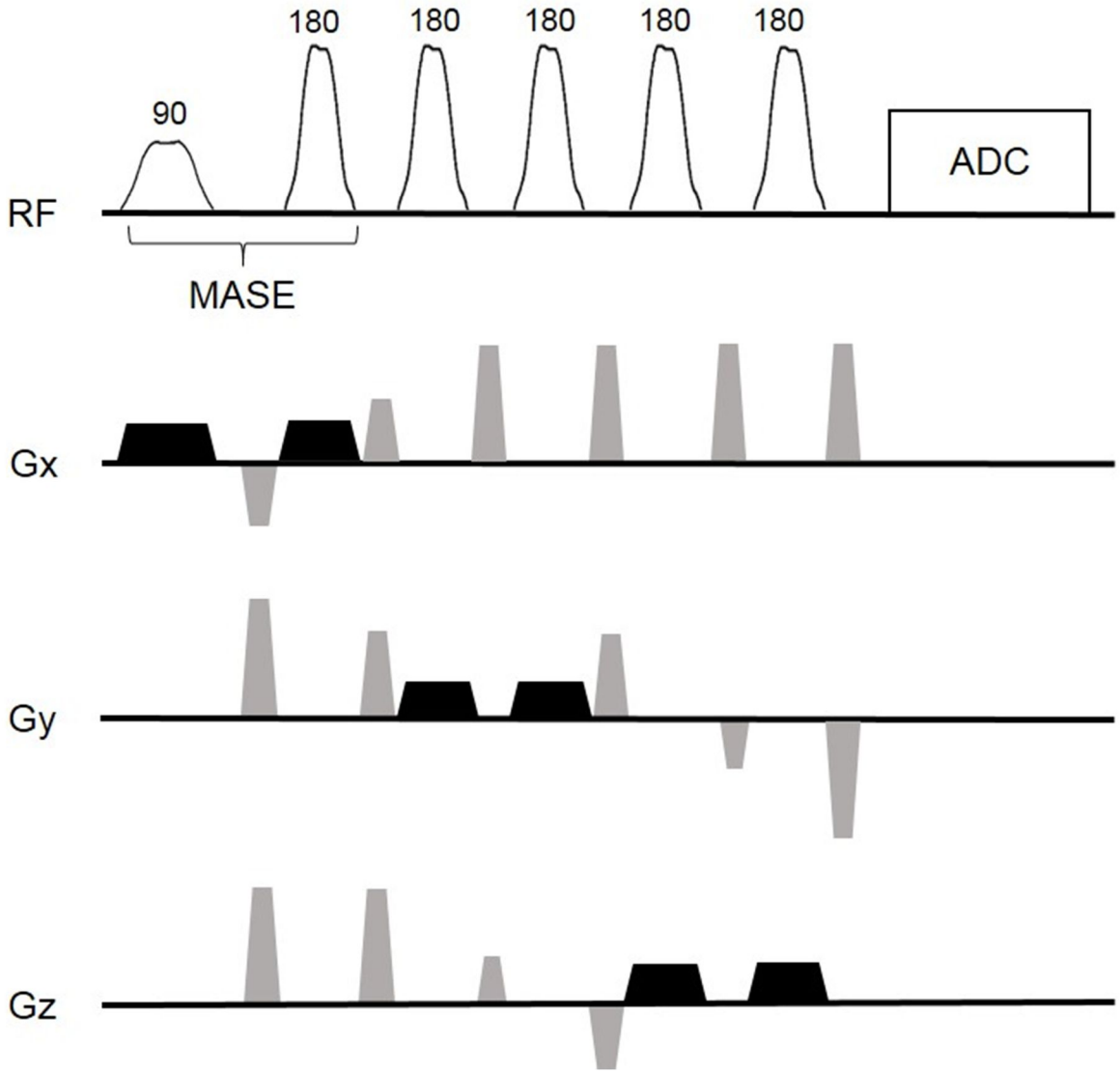


*Figure 1: MASE-sLASER sequence implemented using MASE and two pairs of MASE adiabatic SLR refocusing pulses for short TE = 27 ms SVS acquisition at 7T. MASE pair are used for slice selection in one direction. Two pairs of adiabatic SLR refocusing pulses of the MASE are used for slice selection in the two other directions. The implemented MASE-sLASER sequence therefore consists of 6 RF pulses compared with 5 RF pulses in sLASER and 7 RF pulses in full LASER. Black trapezoids show slice selection gradients and gray trapezoids represent spoiler gradients that are used to dephase unwanted echoes.*

The same MASE RF pulses introduced in Dyvorne et al[14] were used here. All parameters for the design of these pulses are as given in the publication by Dyvorne et al[14].

A 3.5 ms SLR excitation pulse with 5 kHz bandwidth and a 1.75 ms adiabatic SLR refocusing pulse with 4.63 kHz bandwidth were used. Two pairs of the same adiabatic SLR refocusing pulses with 1.75 ms duration and 4.63 kHz bandwidth were used for slice selection in the two other directions. We used a combination of orthogonal spoiler gradients in the sequence to dephase unwanted echoes. The spoiler gradients have 25 mT/m amplitude and their duration varies between 1.2 to 2.6 ms. With this combination of RF pulses, slice selection and spoiler gradients, we implemented a sLASER sequence with a minimum TE of 27 ms and matched CSDE of 1.25 mm/ppm for a voxel size of 20x20x20 $mm^3$ (approximately 6%). Four RF-pulse WET water suppression with less sensitivity to $B_1$ variations than three RF WET [16] was used prior to the localization sequence.

## MEGA-sLASER (with MASE) with TE = 68 ms

In this study, we implemented MEGA-sLASER (with MASE) sequence with TE = 68 ms at 7T (Figure 2).

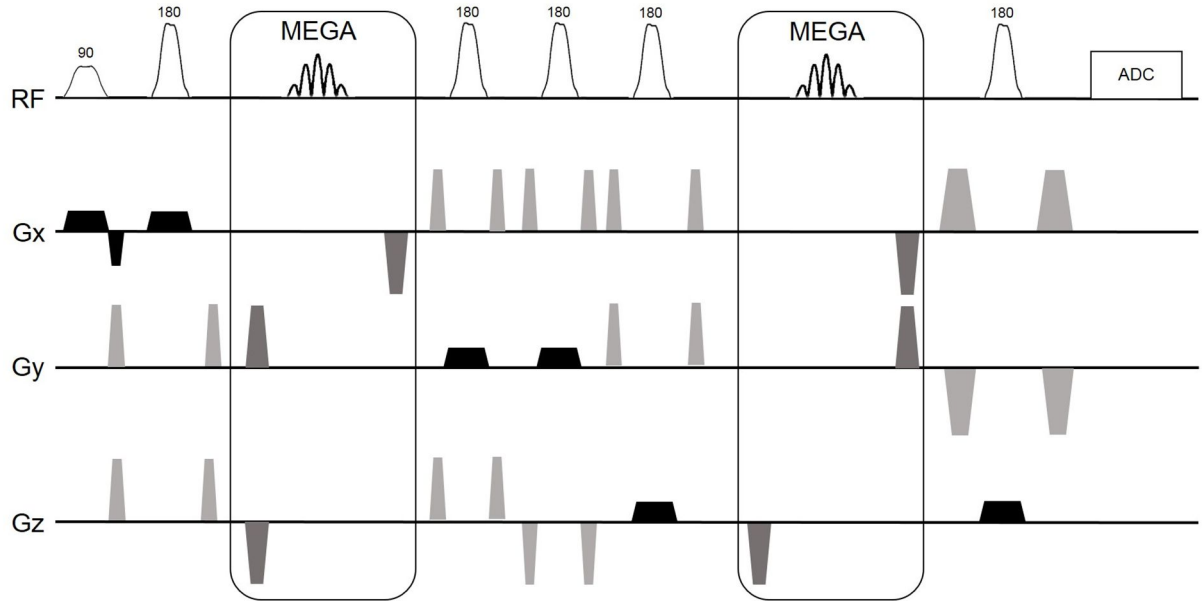


*Figure 2: MEGA-sLASER (with MASE) sequence implemented using MASE and two pairs of MASE adiabatic SLR refocusing pulses with TE = 68 ms for editing GABA at 7T. MEGA pulses are dual band and are used for editing GABA and extra water suppression. Black trapezoids show slice selection gradients, light gray trapezoids represent spoiler gradients used to dephase unwanted echoes and dark gray trapezoids represent MEGA editing gradients that are used to suppress residual water signal.*

For the localization part of the sequence, MASE pulses were used. A 5 ms SLR excitation pulse with 3.52 kHz bandwidth and a 2.5 ms adiabatic SLR refocusing pulse with 3.24 kHz bandwidth were used. Two pairs of the same adiabatic SLR refocusing pulses with 2.5 ms duration and 3.24 kHz bandwidth were used for slice selection in the two other directions. These pulse durations were sufficient to attain the 68ms TE, and shorter pulses were not necessary, as CSDE is not an issue in MEGA editing. Besides, a pair of dual band inversion pulses with a bandwidth of 220 Hz and duration of 11.52 ms were used for MEGA editing and extra water suppression. MEGA-ON and MEGA-OFF modes are interleaved in the sequence as even and odd acquisitions with MEGA pulse excitation centered at 1.9 ppm and 4.7 ppm in ON mode and 4.7 ppm and 7.5 ppm in OFF mode respectively. Here we also used an orthogonal scheme for spoiler gradients with 25 mT/m amplitude and durations between 0.8 to 2 ms. The same WET water suppression was used as above.

## GABA at 2.28 ppm

In preliminary experiments, a putative GABA signal at 2.28ppm was detected. To authenticate this signal two sets of experiments were conducted. In the first the SVS experiment was repeated 9 times, in order to examine whether the signal averaged as a 'signal' component rather than as noise. Furthermore, the MASE-sLASER sequence was also used to acquire spectra at TEs 68 and 136 ms. GABA signal at 2.28 ppm is a triplet coupled to a partner at 1.89 ppm. Its pattern therefore changes as a function of TE because of the effect of J-coupling. The two side peaks of GABA at 2.28 ppm are in in-phase states at TEs 68 and 136 ms. The known pattern of the GABA signal at these two TEs therefore was the reason to choose them to examine the capability of the MASE-sLASER sequence to directly measure GABA at 2.28 ppm. We also performed simulations to examine the pattern of GABA triplet at 2.28 ppm and its neighboring Glutamate multiplet at 2.35 ppm when acquired with the MASE-sLASER and conventional sLASER sequences at TEs 68 and 136 ms. Two parametric spectral models for GABA and Glutamate were simulated using the NMRSIM module of the TOPSPIN suite (Version 3.6, Bruker, Rheinstetten, Germany) with the same sequence and scan parameters as the in-vivo spectroscopy scan. To compare MASE-sLASER and conventional sLASER, we acquired spectra at TE = 68 ms and 136 ms using MASE-sLASER and conventional sLASER in two separate consecutive acquisitions from the same subject and the same voxel. Matched CSDE for the

MASE-sLASER sequence with TE = 68 ms was 1.79 mm/ppm for a voxel size of 20x20x20 $mm^3$ (approximately 9%).

There are two groups of glutamine peaks positioned between 2.10-2.16 ppm and 2.39-2.50 ppm which are well separated from the GABA peak at 2.28 ppm, which was the reason that Glutamine was not included in the simulations.

## Data acquisition

In total, 8 healthy subjects (4 male; age 26.5±3.2 years) participated in this study, with approval from the local ethics committee. In vivo scans were performed on a 7T system (Magnetom 7T, SIEMENS Healthcare GmbH, Germany) with 32 channel Rx and single channel Tx head coil (Nova Medical, NY). An anatomical reference image was acquired using 3D MP-RAGE ([17]) (256 slices, slice thickness = 1 mm, TR = 2500 ms, TE = 1.35 ms, TI = 1100 ms, Flip angle = 6°, FOV = 256 x 256 x 256 mm, 256 x 256 acquisition matrix, GRAPPA acceleration factor 2 PE, Ref. lines PE = 48, phase partial Fourier = 6/8, slice partial Fourier = 6/8, scan duration = 298 s). $B_0$ shimming was performed using FASTESTMAP [18].

Single voxel MRS data were collected from a 20x20x20 $mm^3$ voxel positioned at the medial occipital region of two subjects (subjects 1 and 2) using the short TE MASE-sLASER sequence (TR=4500 ms, TE=27 ms, NEX = 64, scan time=5:06 mins). Single voxel MRS data were also collected from a 20x20x20 $mm^3$ voxel positioned at the medial occipital region of five subjects (subjects 4 to 8) using the MEGA-sLASER (with MASE) sequence (TR=4500ms, TE=68ms, NEX = 64, scan time = 5:06 mins).

To show the feasibility of direct measurement of GABA at 2.28 ppm, we acquired nine consecutive MASE-sLASER spectra at TE = 68 ms from a voxel size of 20x20x20 $mm^3$ positioned at the medial occipital region of a subject (Subject 3) (TR=4500 ms, TE=68 ms, NEX = 32, scan time=2:42 mins).

We examined the separation of the 2.28 ppm GABA line from its neighboring 2.35 ppm Glu line at TEs 68 and 136 ms *in vivo* using the MASE-sLASER sequence and the sLASER sequence. Specifically, we collected spectra from a 20x20x20 $mm^3$ voxel positioned at medial occipital region of a subject (Subject 5) (TR=4500ms, TE=68ms, NEX = 64, scan time = 5:06 mins for both sequences). Also, we collected spectra using the MASE-sLASER sequence and conventional

sLASER sequence from the same voxel size and region of two subjects (Subjects 1 and 2) (TR=4500ms, TE=136ms, NEX = 64, scan time = 5:06 mins for both sequences).

The medial occipital region is a benign region for MRS. To compare the performance of the MASE-sLASER sequence and its sensitivity for GABA measurement with the conventional sLASER sequence, we compared the two sequences with the same TE = 38 ms at six different regions of the brain. The reason why we compared the two methods at TE = 38 ms was that this was the minimum TE of the sLASER sequence. We collected spectra from voxels of size 20x20x20 $mm^3$ positioned in: anterior cingulate, dorsolateral prefrontal cortex, motor cortex, precuneus, posterior cingulate and occipital cortex. The spectra were acquired using MASE-sLASER and conventional sLASER from a healthy subject (Subject 5) (TR=4500ms, TE=38ms, NEX = 64, scan time = 5:06 mins for both sequences). These regions are representative of those accessible to SVS at 7T, primarily because of the poor $B_0$-homogeneity of more inferior regions.

In table 1, we summarize the application and goal of each acquisition, also that which subject(s) were used for which application, also gender and age of the subjects.

| Application | Goal | Subject Number | Gender/Age |
|---|---|---|---|
| MASE-sLASER (TE = 27 ms) | Demonstrate MASE-sLASER sequence as a short TE SVS sequence. | 1 and 2 | two female/25±1.4 years |
| Nine consecutive acquisitions with MASE-sLASER (TE = 68 ms) | Show that signal at 2.28 ppm is not noise. | 3 | male/29 years |
| MEGA-sLASER [with MASE] (TE = 68 ms) | Demonstrate MEGA-sLASER [with MASE] sequence at TE = 68 ms. | 4 to 8 | three male/26.6±3.8 years |
| Comparison between MASE-sLASER and sLASER (TE = 68 ms) | Show feasibility of measuring GABA at 2.28 ppm with MASE-sLASER (TE = 68 ms). Compare with standard sLASER at same TE. | 5 | male/27 years |
| Comparison between MASE-sLASER and sLASER (TE = 136 ms) | Same as above at TE = 136ms. | 1 and 2 | two female/25±1.4 years |
| Comparison between MASE-sLASER and sLASER in six regions (TE = 38 ms) | Compare spectral quality and absolute concentration over a range of conditions. | 5 | male/27 years |

*Table 1: A summary of the application and goal of each acquisition and that which subject(s) were used for which applications.*

TR in all acquisitions was 4500 ms which was long enough to avoid SAR limitations. To have an estimation of relative SAR for the two sequences of sLASER and MASE-sLASER, the ratio of $\sum B_1^2$ was calculated for a typical reference voltage of 230 V. The $\sum B_1^2{}_{\text{MASE-sLASER}} / \sum B_1^2{}_{\text{sLASER}}$ ratio calculated in this way was 0.45.

Data were analyzed using LCModel [19], JMRUI [20] and MATLAB (version. 2016b, Natick, MA).

LCModel software (Version 6.3-1L, Stephen Provencher, Ontario, Canada) [19] was used to quantify the measured metabolites in this study. The basis set for the LCModel analysis of the short TE spectra consisted of twenty-one simulated metabolites. For the MEGA editing method, six edited metabolites of GABA, Glu, Gln, NAA, NAAG and GSH were included in the basis set. The metabolite concentrations were estimated and the Cramér–Rao lower bound (CRLB) expressed in %SD. From twenty-one simulated metabolites of the short TE spectra, and six simulated metabolites of the MEGA method, the major ones are presented in tables 2-5. For the absolute quantification of the metabolites presented in table 2, unsuppressed water spectra were also acquired with MASE-sLASER and sLASER sequences at TE = 38 ms.

To demonstrate the separation of GABA at 2.28 ppm from the neighboring Glutamate at 2.35 ppm with the MASE-sLASER sequence, we also scanned a brain phantom containing NAA (12 mmol/l), Glu (10 mmol/l), Cr (8 mmol/l), Gln (4 mmol/l), m-Ins (5 mmol/l), GABA (2 mmol/l), Asp (2 mmol/l) and Cho (2 mmol/l). The spectra were acquired at TEs 68 ms and 136 ms with the same parameters as in vivo.

# Results

Figure 3 shows the pulse shapes and profiles of the MASE pulses used for excitation in the implementation of the MASE-sLASER sequence and of the conventional SLR pulse used for excitation in the implementation of the sLASER sequence.

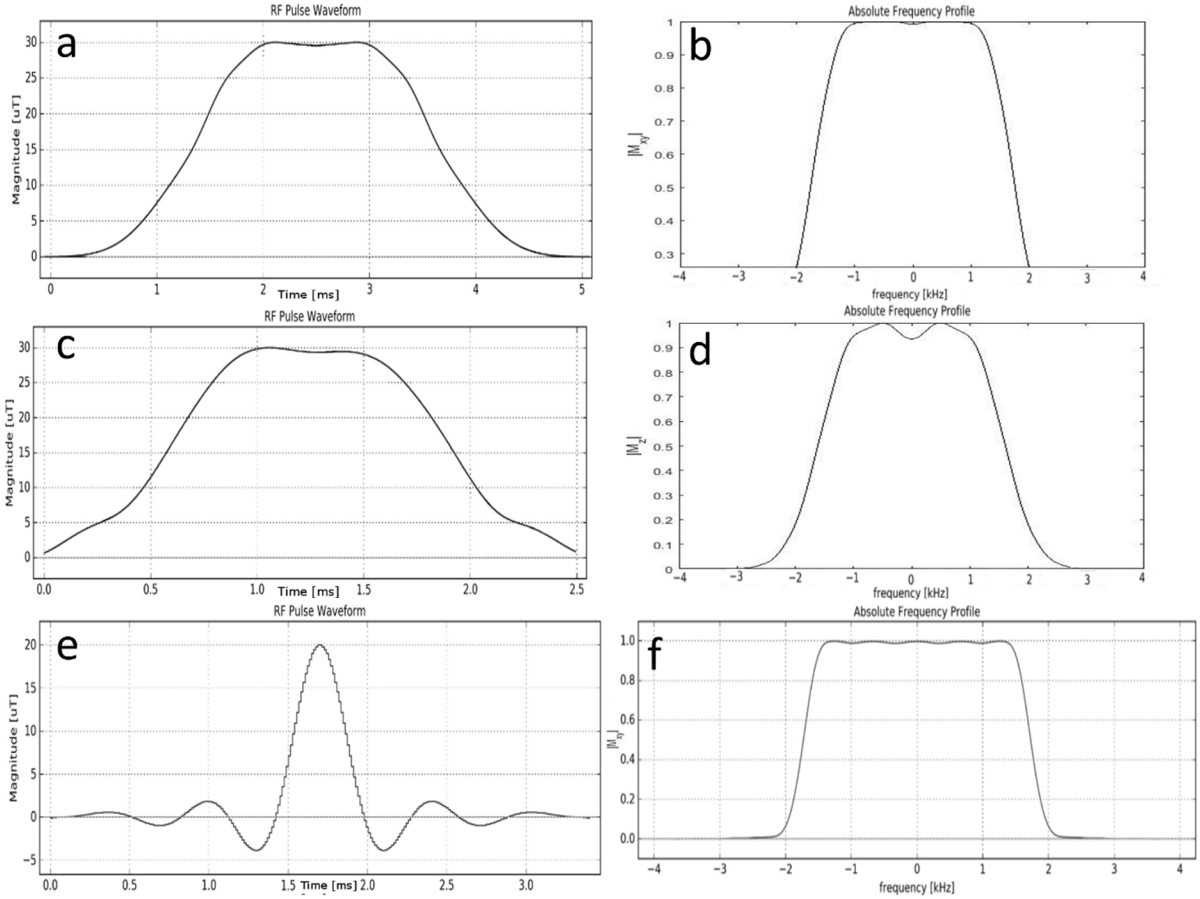


*Figure 3: Pulse shape and frequency response of the excitation parts of MASE-sLASER and sLASER sequences. Excitation part of the MASE-sLASER sequence consists of a MASE spin echo and excitation part of the sLASER is a conventional SLR pulse. a and b are pulse shape and frequency response of the 90 pulse of the MASE respectively, c and d are pulse shape and frequency response of the 180° pulse of the MASE respectively, e and f are pulse shape and frequency response of the conventional SLR excitation pulse used in the implementation of the conventional sLASER sequence.*

In Figure 4, we show a comparison of the CSDE for the MASE-sLASER and sLASER sequences. The CSDE is matched in all three directions and is symmetrical for the MASE-sLASER sequence.

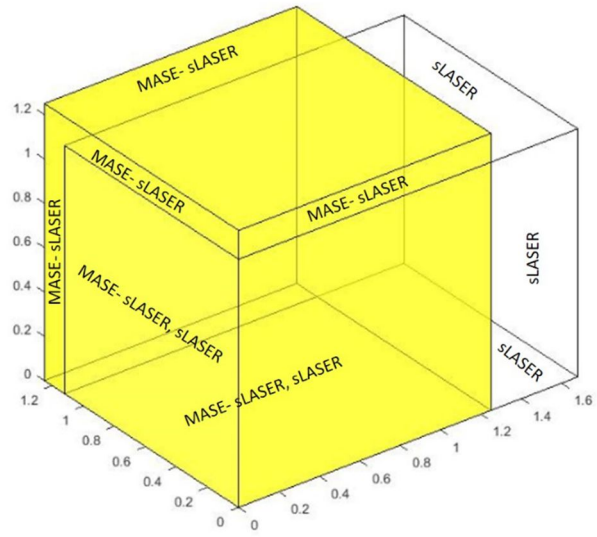


*Figure 4: CSDE for the implemented short TE MASE-sLASER sequence compared with a conventional sLASER sequence. CSDE for MASE-sLASER sequence is symmetric in all three direction (yellow). CSDE for the conventional sLASER is not symmetric and is larger in one direction (white). The cubes here do not represent the voxel, they only show the amount of CSDE in three directions in mm/ppm for each sequence.*

A short TE = 27 ms spectrum acquired from a 20x20x20 $mm^3$ voxel positioned at the medial occipital region of a healthy subject using the MASE-sLASER sequence is shown in Figure 5. Major observed metabolites are labeled in the spectrum. Another MASE-sLASER spectrum acquired with the same acquisition parameters from another subject is provided in the supplementary materials (Supplementary figure 1).

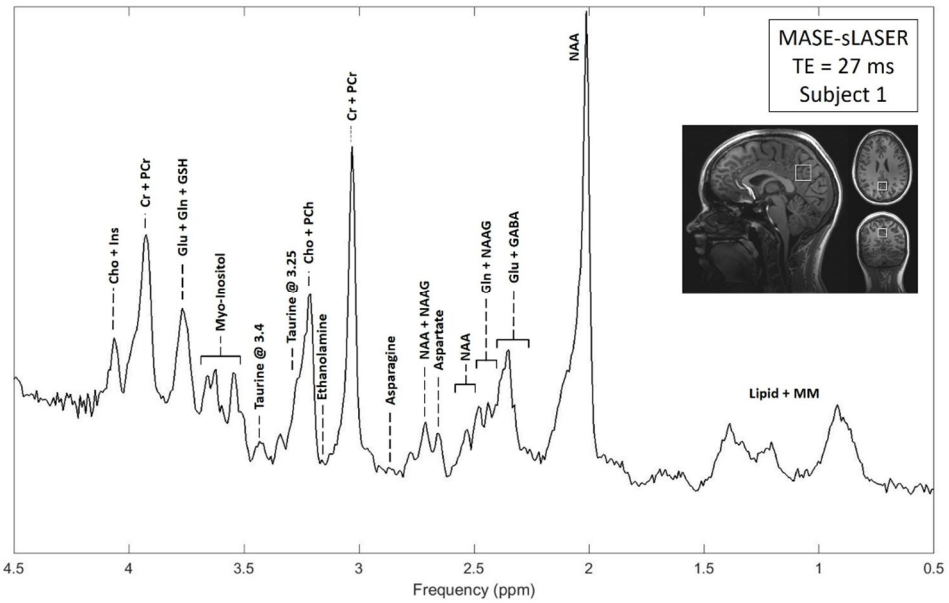


*Figure 5: Short TE MASE-sLASER single voxel spectrum acquired from a 20x20x20 mm$^3$ voxel positioned at the medial occipital region of the human brain at TE = 27 ms at 7T. Major observed metabolites are labeled in the spectrum.*

MEGA difference spectra showing GABA+ at 3 ppm and Glx at 3.75 ppm acquired from a 20x20x20 mm$^3$ voxel positioned at the medial occipital region of a healthy subject using MEGA-sLASER (with MASE) sequence is shown in Figure 6. Four additional MEGA-sLASER (with MASE) spectra acquired with the same acquisition parameters from four subjects are provided in the supplementary materials (Supplementary figure 2).

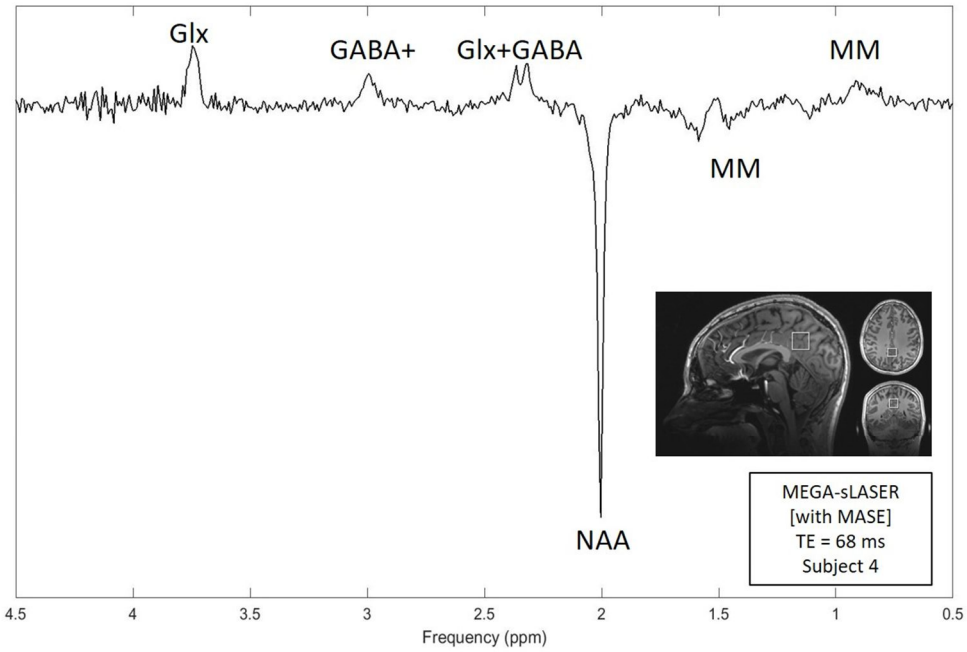


*Figure 6: MEGA difference spectrum acquired using the MEGA-sLASER (with MASE) sequence implemented with MASE pulses at TE = 68 ms from a 20x20x20 $mm^3$ voxel positioned at the medial occipital region of the brain of a healthy subject at 7T showing GABA+ at 3 ppm and Glx at 3.75 ppm. In addition to GABA and Glx, NAA and MM are also coedited in the MEGA edited spectrum.*

The absolute concentration of the major metabolites measured in six different regions of the brain of a healthy subject (5) using sLASER and MASE-sLASER sequences with the same TE = 38 ms and their corresponding CRLB values are shown in table 2. There is a good agreement between the absolute concentration of all metabolites across the two methods, however GSH, GABA and Gln show differences. The results suggest marginal differences between the sensitivity of the two sequences.

| Metabolite | AC | | | | DLPFC | | | | MC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration [mMol/Kg] | | CRLB(%) | | Concentration [mMol/Kg] | | CRLB(%) | | Concentration [mMol/Kg] | | CRLB(%) | |
| | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER |
| **GSH** | **1.48** | **2.37** | **8%** | **5%** | **1.777** | **2.416** | **7%** | **5%** | **1.41** | **2.29** | **10%** | **6%** |
| NAA | 5.95 | 5.56 | 2% | 2% | 7.26 | 6.76 | 2% | 2% | 7.59 | 7.41 | 2% | 2% |
| NAAG | 0.463 | 0.647 | 16% | 13% | 0.822 | 0.86 | 10% | 11% | 0.924 | 0.993 | 10% | 10% |
| mI | 5.307 | 4.36 | 4% | 5% | 5.032 | 4.8 | 5% | 6% | 5.16 | 4.92 | 5% | 6% |
| **GABA** | **0.05** | **2.67** | **663%** | **17%** | **0.491** | **2.88** | **80%** | **19%** | **0.277** | **2.44** | **157%** | **23%** |
| **Gln** | **0.487** | **2.38** | **23%** | **17%** | **0.447** | **1.293** | **28%** | **33%** | **0.142** | **0.53** | **103%** | **82%** |
| Glu | 9.33 | 8.2 | 4% | 4% | 8.121 | 6.567 | 5% | 5% | 7.42 | 7.13 | 6% | 5% |
| Cho+GPC+Pch | 1.276 | 1.36 | 3% | 3% | 1.39 | 1.62 | 4% | 6% | 1.35 | 1.45 | 4% | 3% |
| Cr + PCr | 6.185 | 6.18 | 1% | 2% | 6.262 | 6.468 | 2% | 2% | 6.32 | 6.78 | 2% | 2% |

| Metabolite | Prc | | | | PC | | | | Occ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration [mMol/Kg] | | CRLB(%) | | Concentration [mMol/Kg] | | CRLB(%) | | Concentration [mMol/Kg] | | CRLB(%) | |
| | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER | sLASER | MASE-sLASER |
| **GSH** | **1.8** | **2.625** | **6%** | **5%** | **1.76** | **2.29** | **6%** | **7%** | **1.72** | **2.51** | **7%** | **6%** |
| NAA | 7.6 | 7.2 | 1% | 2% | 7.54 | 7.31 | 2% | 2% | 8.04 | 7.85 | 1% | 2% |
| NAAG | 0.65 | 0.555 | 12% | 17% | 0.72 | 0.69 | 12% | 17% | 0.674 | 0.584 | 14% | 20% |
| mI | 4.62 | 4.148 | 4% | 6% | 4.65 | 4.51 | 5% | 7% | 4.4 | 4.075 | 5% | 8% |
| **GABA** | **0.967** | **2.296** | **40%** | **22%** | **0.78** | **2.31** | **51%** | **24%** | **1.12** | **1.65** | **38%** | **32%** |
| **Gln** | **0.591** | **1.218** | **19%** | **33%** | **0.49** | **1.43** | **26%** | **35%** | **0.543** | **0.702** | **23%** | **71%** |
| Glu | 9.94 | 8.476 | 4% | 4% | 9.92 | 9.54 | 5% | 4% | 8.93 | 8.904 | 5% | 5% |
| Cho+GPC+Pch | 0.87 | 0.952 | 4% | 5% | 0.94 | 1.16 | 4% | 5% | 0.68 | 0.664 | 5% | 9% |
| Cr + PCr | 6.55 | 6.8 | 1% | 2% | 6.78 | 7.26 | 1% | 2% | 6.495 | 7.057 | 2% | 2% |

*Table 2: A comparison of the absolute concentration of the metabolites measured with MASE-sLASER and sLASER sequences at TE = 38 ms from six regions of the brain of a healthy subject (5).*

Table 3 shows a comparison of the concentration of all the metabolites measured with the MASE-sLASER sequence at TE = 27 ms from subjects 1 and 2.

| **MASE-sLASER, TE = 27 ms** | | | | |
|---|---|---|---|---|
| Metabolite | Subject 1 | | Subject 2 | |
| | Conc. (Met./tNAA) | CRLB | Conc. (Met./tNAA) | CRLB% |
| GSH | 0.332 | 5% | 0.297 | 6% |
| NAA | 0.929 | 2% | 0.924 | 2% |
| NAAG | 0.07 | 19% | 0.076 | 17% |
| mI | 0.758 | 4% | 0.676 | 4% |
| GABA | 0.49 | 11% | 0.494 | 11% |
| Gln | 0.269 | 16% | 0.297 | 13% |
| Glu | 1.257 | 3% | 1.195 | 3% |
| Cho+GPC+Pch | 0.134 | 5% | 0.145 | 4% |
| Cr + PCr | 1.03 | 2% | 1.034 | 2% |

*Table 3: A comparison of the concentration of all the metabolites measured with MASE-sLASER sequence at TE = 27 ms from subjects 1 and 2. The metabolites are quantified with LCModel.*

Table 4 shows a comparison of the concentration of metabolites measured with MEGA-sLASER (with MASE) sequence at TE = 68 ms from subjects 4 to 8.

| MEGA-sLASER [with MASE], TE = 68 ms | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Metabolite | Subject 4 | | Subject 5 | | Subject 6 | | Subject 7 | | Subject 8 | | mean ± std | |
| | Conc. (Met./tNAA) | CRLB% | Conc. (Met./tNAA) | CRLB% | Conc. (Met./tNAA) | CRLB% | Conc. (Met./tNAA) | CRLB% | Conc. (Met./tNAA) | CRLB% | Conc. (Met./tNAA) | CRLB% |
| GABA | 0.112 | 19% | 0.08 | 25% | 0.08 | 25% | 0.102 | 22% | 0.113 | 18% | 0.097±0.016 | 21.8±3.27% |
| Gln | 0.188 | 34% | 0.08 | 87% | 0.08 | 87% | 0.023 | 322% | 0 | 999% | 0.093±0.069 | 305.8±403% |
| Glu | 0.961 | 8% | 0.886 | 9% | 0.886 | 9% | 0.96 | 9% | 0.962 | 6% | 0.931±0.041 | 8.2±1.3% |

*Table 4: A comparison of the concentration of the metabolites measured with MEGA-sLASER (with MASE) sequence at TE = 68 ms from subjects 4 to 8. The metabolites are quantified with LCModel.*

In table 5 we show a comparison of the concentration of the major metabolites of NAA, tCho and tCr measured with sLASER and MASE-sLASER at TE = 38 ms in this study, with concentrations reported in Ganji et al[13] for the medial frontal lobe and medial occipital lobe. There is a good agreement between the quantification of the major metabolites (NAA, tCho and tCr) across the three measurements (sLASER, MASE-sLASER and PRESS [Ganji et al[13]]).

| Metabolite | Medial Frontal Lobe | | | Medial Occipital Lobe | | |
|---|---|---|---|---|---|---|
| | Concentration (ratio to tNAA) | | | Concentration (ratio to tNAA) | | |
| | sLASER | MASE-sLASER | Ganji et al [13] | sLASER | MASE-sLASER | Ganji et al [13] |
| NAA | 0.86 | 0.87 | 0.924 | 0.89 | 0.95 | 0.88 |
| tCho | 0.18 | 0.22 | 0.181 | 0.11 | 0.14 | 0.113 |
| tCr | 0.92 | 1.04 | 0.82 | 0.82 | 0.93 | 0.755 |

*Table 5: A comparison of the concentration of major metabolites (NAA, tCho and tCr) measured with sLASER and MASE-sLASER sequences in this study with Ganji et al[13] in medial occipital and medial frontal lobe of the healthy human brain.*

A notable feature of MASE-sLASER spectra at TEs of 68ms and 136 ms is that signal at 2.28 ppm is well separated from the major neighboring Glutamate signal at 2.35 ppm. The simulation results for GABA and Glutamate are shown in Figures 7 (a, c, e, g). The simulation is performed for these two metabolites and for MASE-sLASER and conventional sLASER sequences at TEs 68 and 136 ms, the echo times at which the pattern of the GABA triplet at 2.28 ppm falls in the real channel. An interesting feature of the simulated spectra is that these two molecules' resonances centered at 2.28 ppm and 2.35 ppm are in-phase relative to each

other at TEs of 68 and 136 ms when acquired with the MASE-sLASER sequence (Figures 7(a, e)), but not for the conventional sLASER sequence (Figures 7(c, g)). For MASE-sLASER the only overlapping sub peak of Glutamate with GABA signal is the peak number 3 (upfield) which is in phase, relative to the coincident GABA resonance at TEs 68 and 136 ppm. Considering this peak of the Glutamate as being very small in comparison to the two other peaks, we conclude that the contribution of Glutamate to GABA signal is minimal at these two TEs and therefore, GABA at 2.28 ppm and Glutamate at 2.35 ppm are well separated when acquired by MASE-sLASER sequence, at these two TEs. However, for conventional sLASER peak 2 of Glutamate and peak 1 of GABA overlap at TE = 68 ms (Figure 7c) with peak 2 of Glutamate being much larger than peak 1 of GABA. Also peak 3 of Glutamate overlaps with peak 2 of GABA at this TE. Moreover, peak 2 of Glutamate and peak 1 of GABA totally overlap at TE = 136 ms (Figure 7g). The simulation results therefore, demonstrate a good separation of GABA at 2.28 ppm and Glutamate at 2.35 ppm when acquired with MASE-sLASER at TEs 68 and 136 ms and overlap of these two signals when acquired with the conventional sLASER sequence at the same TEs. In vivo results of MASE-sLASER and conventional sLASER acquisitions at TEs 68 and 136 ms are shown in Figure 7 (b, d, f, h). While GABA and Glutamate signals overlap at TE = 68 ms when acquired with the sLASER sequence (Figure 7d) they are well separated when acquired with MASE-sLASER at the same TE (Figure 7b). Also, while the GABA signal is elevated by the Glutamate signal at TE = 136 ms when acquired with the sLASER sequence (Figure 7h) it is well separated from Glutamate when acquired with the MASE-sLASER sequence at the same TE (Figure 7f). Theoretically, the pattern of the GABA triplet at 2.28 ppm should be negative-positive-negative at TE = 68 ms and positive-positive-positive at TE = 136 ms. The three sub peaks of GABA resonance at 2.28 ppm in vivo measured with MASE-sLASER sequence shown in Figure 7(b, f) have a negative-positive-negative pattern at 68 ms and a positive-positive-positive pattern at 136 ms as expected theoretically and match with the simulated patterns shown in the Figure 7(a, e).

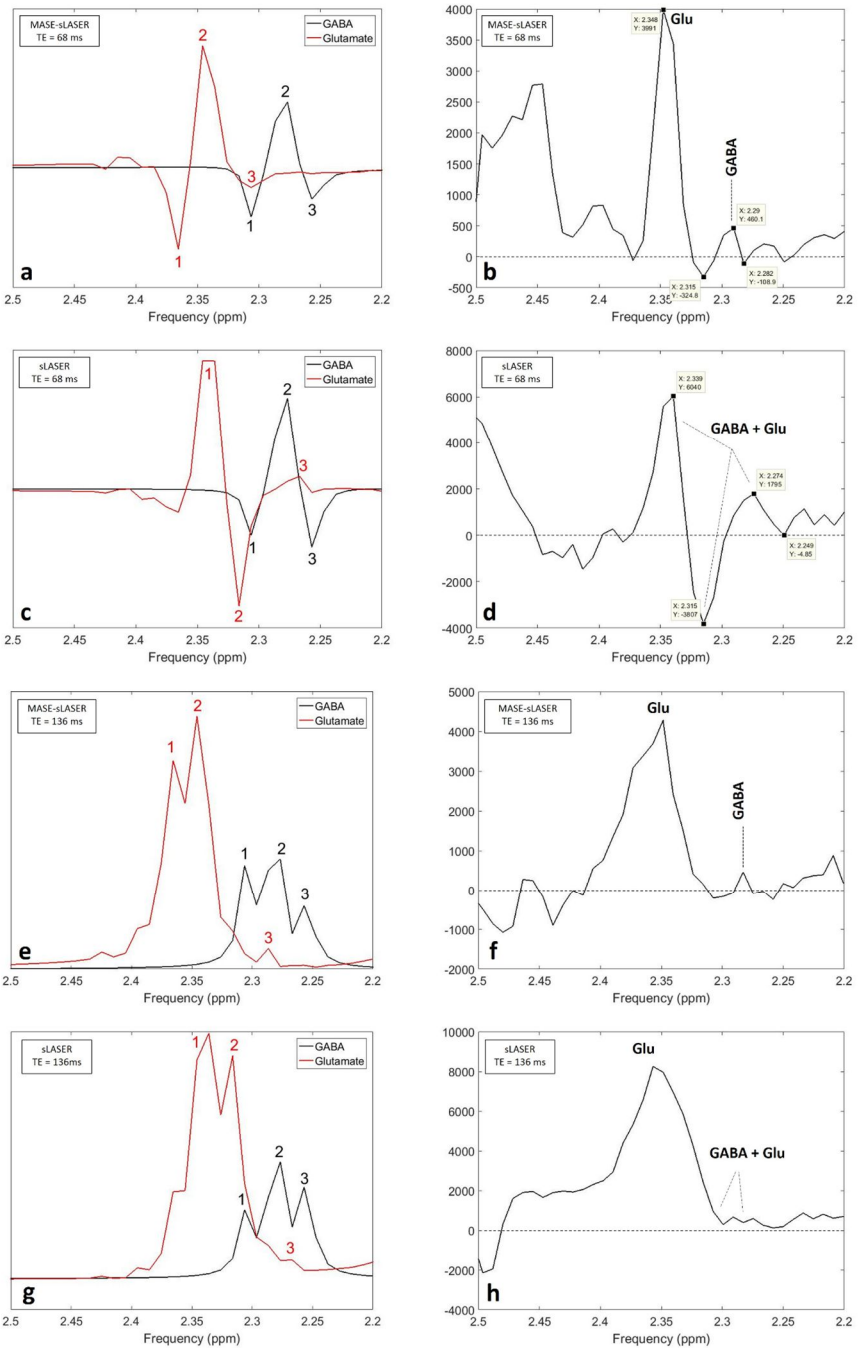


*Figure 7: Simulation for GABA and Glutamate at TE=68 ms for MASE-sLASER (a) and sLASER (c) and at TE = 136 ms for MASE-sLASER (e) and sLASER (g). The major sub-peaks are labeled with numbers 1-3 for each metabolite. Pattern of GABA at 2.28 ppm and Glutamate at 2.35 ppm observed in vivo at TE=68 ms acquired with MASE-sLASER sequence (b) and sLASER (d) and at TE=136 ms acquired with MASE-sLASER sequence (f) and sLASER (h) in good agreement with the corresponding simulation results.*

To show that the signal observed at 2.28 ppm arises from GABA not noise, we also acquired 9 separate consecutive MASE-sLASER data from the same voxel at TE = 68 ms. In Figure 8 we

show the average of the nine acquisitions in the range of 2.1 to 2.5 ppm which presents GABA at 2.28 ppm separated from the neighbor Glutamate at 2.35 ppm. The spectra of the nine single acquisitions in the range of 2.1 to 2.5 ppm are given in the supplementary materials (supplementary figure 3). Due to different possible gain settings of these 9 acquisitions, we first normalized each of these nine spectra based on the average value of their noise (i.e. equal thermal noise level). After alignment of the spectra based on the NAA peak, the SNR of the 2.28 ppm signal for the separate measurements and for the average were calculated. The averaged SNR was on average 3.16 times that of the individual acquisitions confirming that the observed signal is not noise. The only outlier was the second acquisition which comparatively had lower SNR than the other 8 acquisitions.

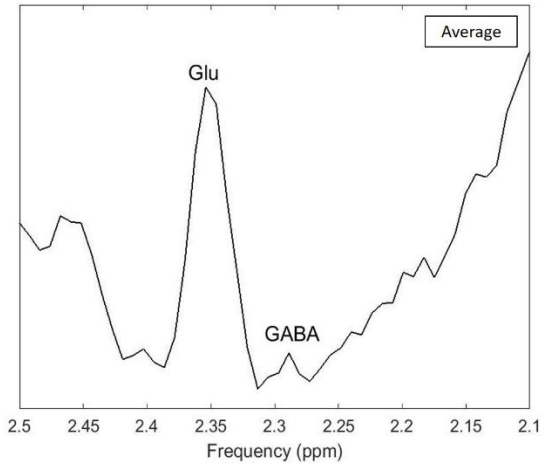


Figure 8: Average spectrum of nine consecutive acquisitions acquired from a 20x20x20 $mm^3$ voxel positioned at the medial occipital region of a healthy subject (Subject 3) using MASE-sLASER sequence with TE = 68 ms at 7T. The spectrum is shown in the range of 2.1 to 2.5 ppm. GABA at 2.28 ppm is clearly separated from Glutamate at 2.35 ppm. The nine individual acquisitions are shown separately in the supplementary materials (supplementary figure 3).

The spectra acquired from a brain phantom at TEs 68 ms and 136 ms using MASE-sLASER sequence are shown in the Figure 9 demonstrating the separation of GABA from Glu at both TEs.

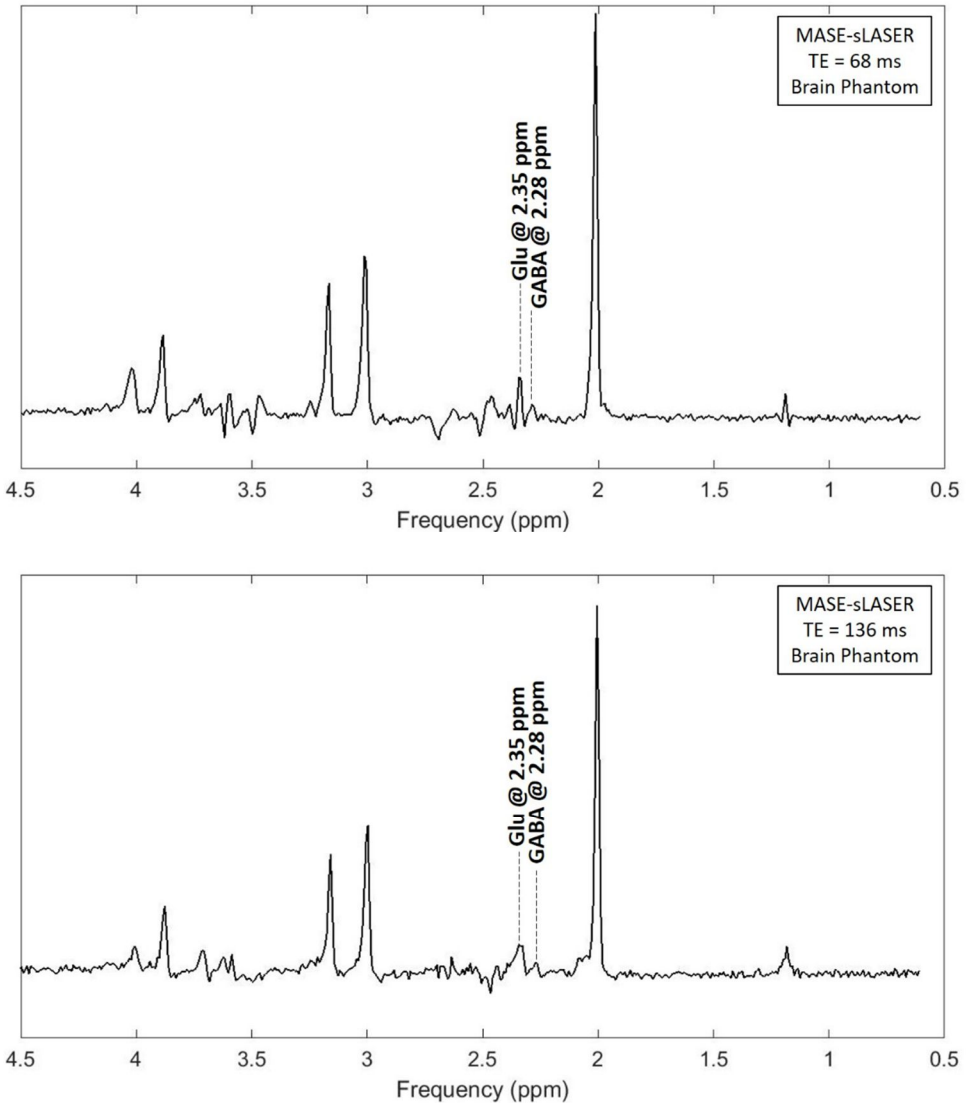


*Figure 9: Spectra acquired using MASE-sLASER sequence from a brain phantom at TEs 68 ms (top) and 136 ms (bottom) showing GABA at 2.28 ppm well separated from Glutamate at 2.35 ppm.*

# Discussion

The main advantage of using adiabatic SLR refocusing pulses in the implementation of MASE-sLASER sequence is their shorter duration compared with the conventional adiabatic pulses. This made it possible to implement the MASE-sLASER sequence here with a TE as short as 27 ms even though we need two pulses for the excitation. Replacing the excitation pulse should bring some reduction in $B_1$ sensitivity, and we match the CSDE in all three directions.

## Short TE MASE-sLASER

The short TE MASE-sLASER matched CSDE sequence is here implemented with minimum TE = 27 ms. As a comparison, the short TE conventional sLASER sequence at 7T for SVS of human brain has been previously implemented at TE = 25 ms [21], TE = 28 ms [15] and TE = 32 ms [22]. Therefore, while our minimum TE is comparable with previous implementations, our sequence however has the advantage of low and matched CSDE in all three directions which until now had been an advantage of full LASER and STEAM sequences and not a feature of the sLASER sequence.

## Comparison with STEAM and standard sLASER

A matched CSDE sequence used for SVS is STEAM. A drawback of this sequences is the halved intensity which is caused by using a stimulated echo. To compensate for this disadvantage, STEAM is usually used at ultrashort TE [23],[24],[25]. In addition to the mentioned drawback, the RF pulses used in the STEAM sequence are not adiabatic which makes the sequence sensitive to $B_1$ inhomogeneity especially at ultrahigh field. Also, these pulses usually have a lower bandwidth than the adiabatic pulses which makes CSDE relatively larger for this sequence. In comparison, MASE-sLASER sequence generates a full intensity echo while keeping the advantage of matched and low CSDE in all three directions. Also, the refocusing pulse used in the MASE pair is adiabatic and therefore, insensitive to $B_1$ variations. The non-adiabatic excitation pulse of MASE has been shown to have lower sensitivity to $B_1$ variations than conventional excitation 90° pulses [14].

There is a drawback for the application of standard sLASER sequence at ultrahigh field which we have compensated for by implementing MASE-sLASER sequence. The standard sLASER sequence has larger and different CSDE in one direction which is because of its non-adiabatic excitation pulse with lower bandwidth than adiabatic pulses. In comparison, MASE-sLASER has low and matched CSDE in all three directions. In fact, CSDE for our implemented short TE MASE-sLASER sequence is 1.25 mm/ppm in all three axes for a voxel size of 20x20x20 $mm^3$ (6.3%). In comparison, our implemented conventional sLASER sequence for the same voxel size has CSDE of 1.7 mm/ppm in non-adiabatic excitation direction (8.4%) and 1.12 mm/ppm in the adiabatic refocusing directions (5.6%).

## MEGA-sLASER (with MASE) with TE = 68 ms for editing GABA

The MEGA J-difference editing technique for GABA requires fixed echo times of odd multiples of 68ms. As higher odd multiples of 68 are generally considered as too long because of the loss in sensitivity caused by T2 relaxation, the optimum echo time for editing GABA is 68 ms [11]. With a large number of pulses of relatively long duration, it is not currently possible to implement a full LASER MEGA editing sequence with echo time of 68 ms at ultrahigh field. To our knowledge, currently there is no report of full LASER for editing GABA at ultrahigh field with TE = 68 ms though GOIA-LASER with TE = 68 ms has been successfully implemented for measuring GABA with MRSI at 3T [26]. While GOIA and MASE pulses are both low power, the major advantage of GOIA pulses relative to MASE pulses is their much higher bandwidth which makes CSDE negligible and thus, makes GOIA pulses more beneficial than MASE for MRSI. For instance, the wide bandwidth of GOIA pulses used in MRSI with LASER makes outer volume suppression of fat unnecessary [27]. On the other hand, implementation of MASE pulses is simpler than that of GOIA pulses, and they can also be used for excitation. Compared to the full LASER sequence, sLASER sequence offers a shorter TE and therefore has been used to efficiently edit GABA at 7T [10]. In this study we present the implementation of MEGA-sLASER (with MASE) sequence with TE = 68 ms at 7T as another application of MASE pulses which is made possible because of the shorter duration of MASE pulses compared with the conventional hyperbolic secant pulses. Previously, MEGA-sLASER at 7T had to be implemented at TEs longer than the optimum value of 68 ms (74 ms [10,15]).

The MEGA editing method implemented here employs two editing inversion pulses that have a narrow bandwidth (220 Hz). These two RF pulses are not adiabatic and therefore, B1 inhomogeneity negatively affects their inversion efficiency. However, the sensitivity of the sequence to variations in the excitation pulse (90°) angle is greater than to variations in the inversion pulse angle.

In the comparison of the concentration of the metabolites presented in tables 3 and 4, there is a good agreement between the calculated values across the subjects for each method which confirms the reproducibility of the two methods of MASE-sLASER and MEGA-sLASER (with MASE).

In the comparison of the concentration of the major metabolites presented in table 5, there is a good agreement between the quantification of the major metabolites (NAA, tCho and tCr) across the three methods (sLASER, MASE-sLASER and PRESS [13] ).

## GABA at 2.28 ppm

The contribution of Glutamate to GABA signal at 2.28 ppm in simulation is negligible at TEs 68 and 136 ms when acquired with the MASE-sLASER sequence (Figure 7(a, e)). On the other hand, simulation results for the conventional sLASER sequence at TEs 68 and 136 ms demonstrate overlap of GABA and Glutamate signals (Figure 7(c, g)).

We have distinguished GABA at 2.28 ppm from Glutamate at 2.35 ppm in the spectra acquired with the MASE-sLASER sequence at TEs of 68 and 136 ms, the echo times at which the side peaks of GABA are in in-phase states because of the effect of J evolution. As shown in figures 7(b, f), the GABA signal is clearly separated from Glutamate at these two TEs. This is in contrast to PRESS acquisitions, where TE = 92 ms was found to be an optimum TE to have GABA and Glutamate well separated [13].

With simulation, the timings of the RF pulses are possible to adjust but it is still difficult to separate timing and pulse effects, for example if we replace the MASE excitation pulse pair with a single SLR pulse then we also need to change the timing of the other pulses. Indeed, there are two main differences between sLASER and MASE-sLASER in terms of sequence elements. The 90° excitation pulse of sLASER is replaced with the MASE pair. In addition to this, the two sequences have different inter-pulse intervals at the same TE. These two factors result in having GABA and Glu separated with MASE-sLASER but not with sLASER at TE = 68 ms. The signal pattern of J coupled metabolites is dependent on the RF pulse scheme and therefore, for sLASER another TE than 68 ms could possibly be optimum to have GABA and Glu separated. However, we have not observed such a separation at the TEs probed in this work. Although a comprehensive search is beyond the scope of this work, we consider it unlikely that such a separation exists at a TE which would also give a good SNR.

For MASE-sLASER specifically, a short TE is not necessarily optimum for the separation of GABA from Glutamate. At TE = 34 ms for instance, the GABA resonance at 2.28 ppm is not clearly separated from the neighboring Glutamate and is not separately visible in vivo, as shown in

supplementary material (supplementary figure 4). Also, in short TE spectra acquired at 27 ms shown in Figure 5 and supplementary materials (supplementary figure 1), GABA peak at 2.28 ppm is not clearly and separately visible.

The main advantages of the MASE-sLASER sequence we are presenting here are separation of the GABA signal at 2.28 ppm from the neighboring Glutamate at 2.35 ppm, and the possibility of implementing MEGA-sLASER (with MASE) with TE = 68 ms to measure GABA at 7T. The CSDE of the MASE-sLASER sequence is comparable with and not much less than that of the conventional sLASER sequence. The improvement in the CSDE of using the MASE-sLASER sequence is limited and in fact the main feature in terms of CSDE is to have a matched CSDE which till now had been a feature of full LASER and not a sLASER sequence.

## Limitations

It is worthwhile to point out that since the excitation 90° pulse used in MASE is not adiabatic, the implemented sequence is not full LASER but sLASER. Though, the only non-adiabatic pulse of the MASE (the excitation 90° pulse) has been shown to have lower sensitivity to $B_1$ variations than the conventional excitation 90° pulses [14]. Even if the improvement in $B_1$ insensitivity is marginal it could have a disproportionate effect on the signal intensity, which is well known to be more affected by imperfections in the excitation than in the refocusing pulses.

A limitation of MASE-sLASER sequence is the lower bandwidth of MASE pulses compared to hyperbolic secant pulses and GOIA pulses. The bandwidths of the MASE pulses used in the implementation of MASE-sLASER sequence in this study are in the range of 4.6-5 kHz which is slightly less than hyperbolic secant pulses used in the implementation of standard sLASER sequence [8,9] and much less than GOIA pulses [27].

# Conclusion

Short TE MASE-sLASER is a full intensity matched CSDE sequence, with a low and matched CSDE in all three directions. Besides, benefiting from the short duration of the MASE pulses, The MEGA-sLASER (with MASE) sequence is implemented here with TE = 68 ms for measuring GABA at 7T. And finally, a characteristic of the spectra acquired with MASE-sLASER at TE=68ms

is the GABA resonance at 2.28 ppm being distinguishable from Glutamate at 2.35 ppm, making direct measurement of this GABA resonance possible with this sequence.

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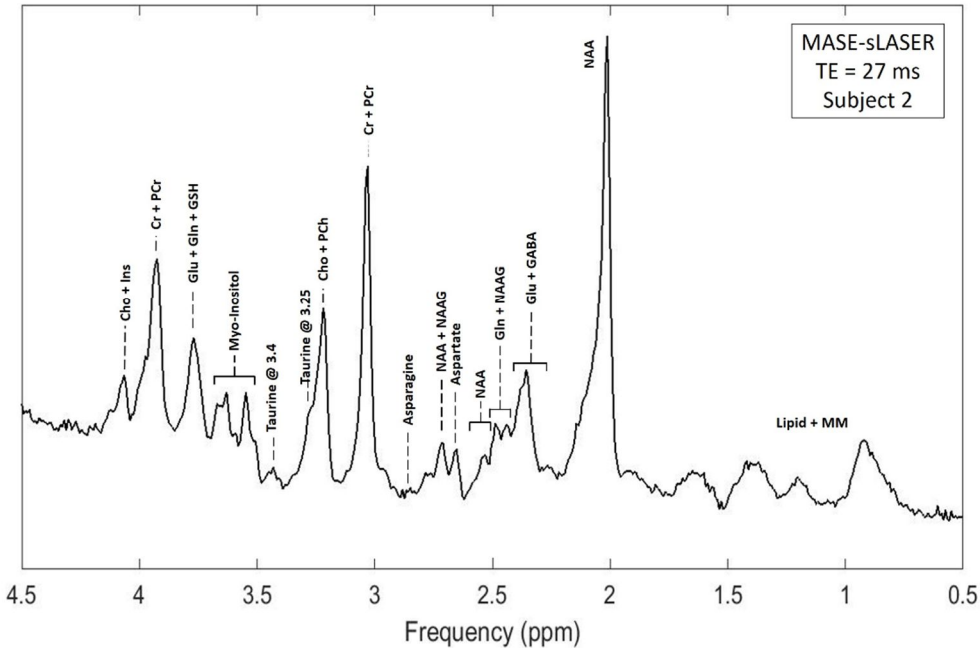
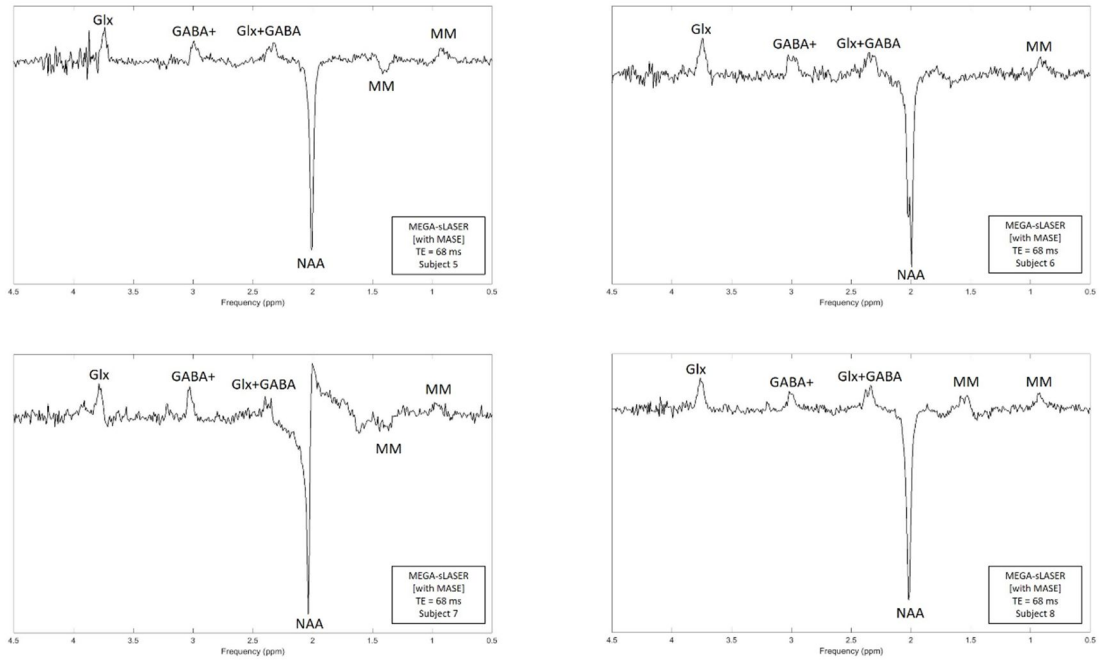
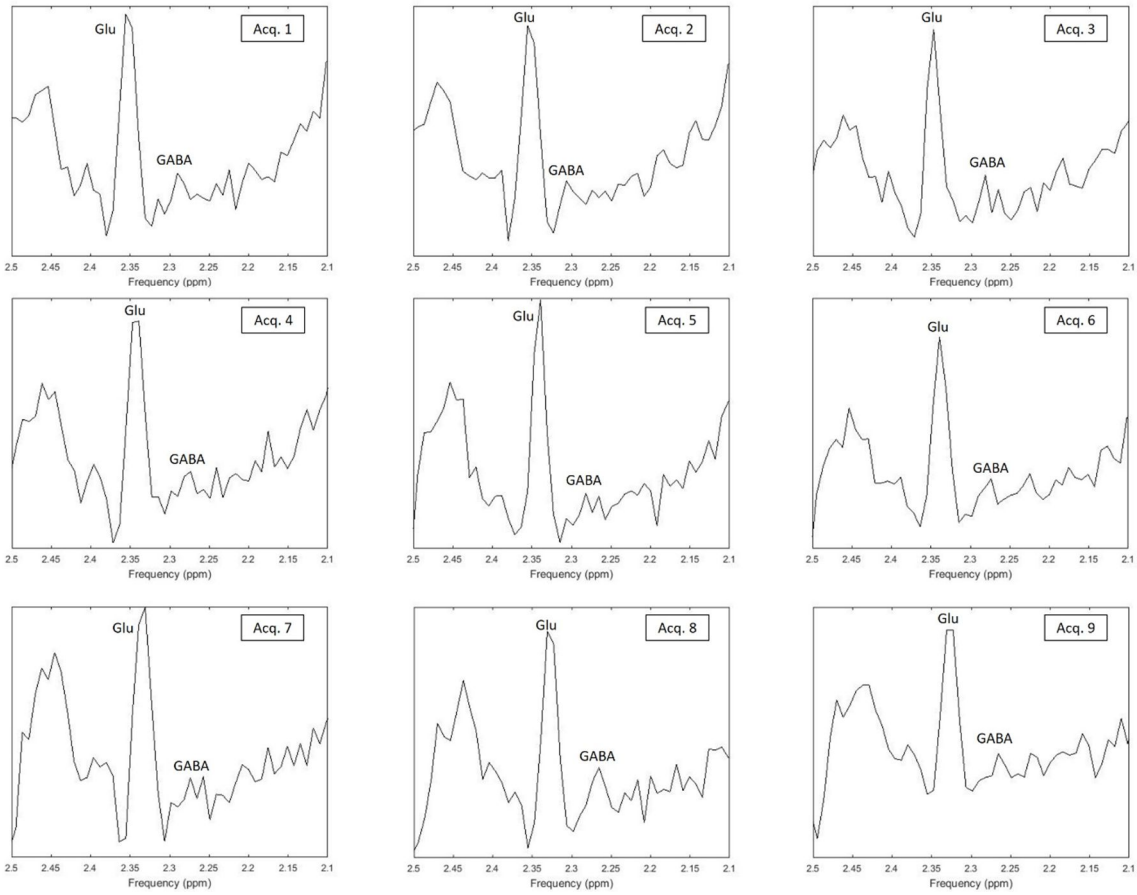
# Supplementary material



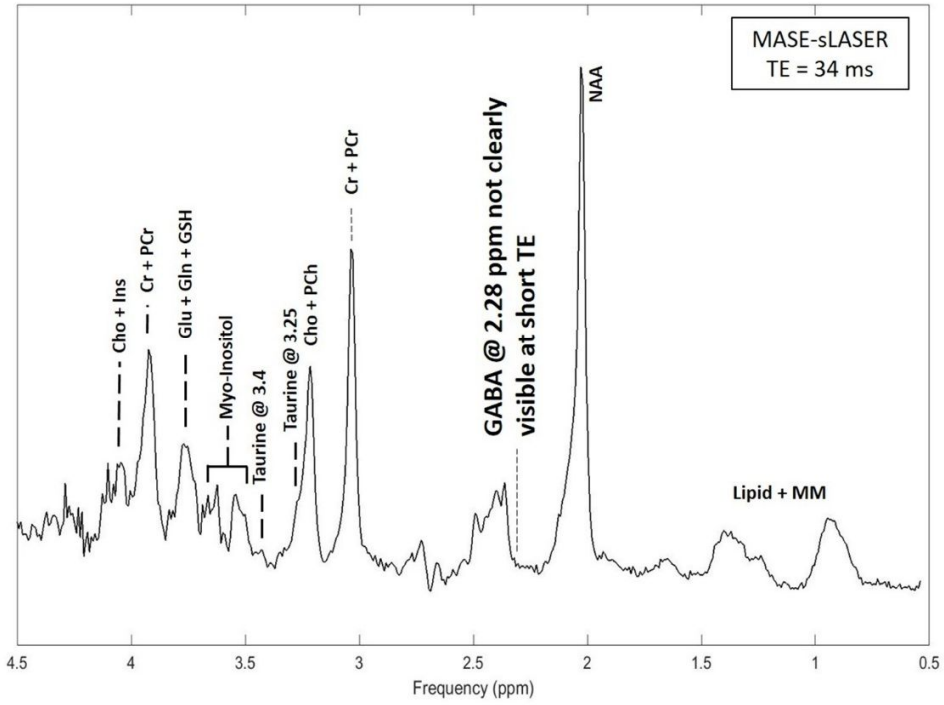

*Supplementary figure 1: MASE-sLASER spectrum obtained at TE =27 ms measured from a 20x20x20 mm$^3$ voxel positioned at the medial occipital region as shown in Figure 5 from a healthy subject (Subject 2) at 7T.*




*Supplementary figure 2: MEGA-sLASER (with MASE) spectra at TE = 68 ms measured from a 20x20x20 mm$^3$ voxel positioned at the medial occipital region as shown in Figure 6 from four healthy subjects at 7T. Measured metabolites are labeled in the spectra.*




Supplementary figure 3: Nine individual MASE-sLASER acquisitions at TE = 68 ms. The spectra are shown in the range of 2.1 to 2.5 ppm. GABA at 2.28 ppm is visible separately from the neighboring Glutamate at 2.35 ppm.




*Supplementary figure 4: MASE-sLASER spectrum acquired at TE = 34 ms. The GABA resonance at 2.28ppm cannot be seen.*

# Chapter 4 - Macromolecule free GABA measurement with MASE-sLASER and MEGA-sLASER at 7T

Seyedmorteza Rohani Rankouhi, Donghyun Hong, David G. Norris

## Abstract

The MEGA editing method is an established MRS approach for measuring GABA in the human brain in vivo. While this method was developed to measure the 3 ppm resonance of GABA isolated from overlapping Creatine using a J-difference principle, it is well known that this signal is contaminated with macromolecules (MM), unless techniques like metabolite nulling with inversion recovery or symmetric inversion in editing (e.g. MEGA) are employed. Contrariwise to GABA resonance at 3 ppm, the GABA resonance at 2.28 ppm is not overlapped by any metabolite, and there is the possibility of direct measurement of this GABA resonance at ultrahigh field, where the neighboring Glutamate at 2.35 ppm is further away from it. We recently demonstrated the possibility of measuring the GABA resonance at 2.28 ppm directly with the MASE-sLASER sequence at TE = 68 ms at 7T. While MEGA and MASE sLASER approaches both acquire spectra at TE = 68 ms, they fundamentally differ in method. We used these two distinct techniques operating at the same TE = 68 ms to acquire spectra from predominantly white matter and predominantly gray matter voxels to gain insight into their ability to measure MM free GABA concentration in human brain at 7T.

## Introduction

Magnetic Resonance Spectroscopy (MRS) is currently the only way to measure GABA in vivo [1,2]. Among several MRS methods introduced to measure GABA, the J-editing method [3] or

MEGA editing method [4] is the most common approach. MEGA was originally introduced as a water suppression method placed inside the localization component of a PRESS sequence. Subsequently, its application was also shown capable of distinguishing the GABA signal from the overlapping Creatine resonance at 3 ppm using a J-coupling based subtraction method [4].

The J-coupling basis of the MEGA editing method brings the danger of coediting other J-coupled metabolites in the edited 3 ppm signal. More specifically, editing is achieved by inverting the coupling partner of 3 ppm GABA at 1.89 ppm. Therefore, any coupled metabolite with a resonance frequency close to 1.89 ppm which is inverted by the editing pulse and has a coupling partner at 3 ppm will contribute to the edited 3 ppm signal. It has long been known that there are macromolecules (MM) that satisfy this condition[5], and hence that the MM signal can be co-edited with GABA, leading to the 3 ppm edited signal often being termed GABA+, with the '+' denoting the additional MM contribution to the signal.

It was recently demonstrated that with the MASE-sLASER sequence at the specific TE of 68 ms, it is possible to measure the GABA resonance at 2.28 ppm well separated from the neighboring Glutamate resonance at 2.35 ppm at 7T [6]. At the same TE, the MEGA editing method is frequently used as an efficient tool to measure the GABA+ resonance at 3 ppm. We have recently implemented the MEGA-sLASER sequence with TE = 68 ms at 7T using MASE pulses[6].

In this study, we will use these two MRS tools to explore the degree to which measured GABA concentrations obtained with these two distinct methods are similar. By using a sufficiently narrow bandwidth editing pulse, we ensure in this study the fully coediting of the GABA line at 2.28 ppm in the MEGA edited spectra. By feeding this signal into an LCModel analysis, we should be able to estimate MM free GABA with the MEGA-sLASER sequence acquisition technique. We will also use LCModel analysis to compare the estimated GABA concentration with the two distinct methods of MASE-sLASER and MEGA-sLASER and in this way will present a validation of direct measurement of 2.28 ppm GABA line with MASE-sLASER sequence. The comparison of the two techniques is under the assumptions: that there is no MM contamination of the 2.28 ppm line at TE = 68 ms; and that the two GABA resonances possess similar T2 relaxation times (which is a reasonable assumption on the basis of the molecular

structure of GABA). We examine here spectra from voxels placed in predominantly gray matter (GM) and predominantly white matter (WM) brain tissue knowing that GABA has a considerably higher concentration in GM than in WM. By plotting GABA concentration as a function of GM/WM concentration we are further able to estimate the relative concentrations of GABA between GM and WM.

# Methods

## Sequence implementation

### MASE-sLASER

MASE-sLASER has previously been implemented using MASE for slice selection in one direction and two pairs of adiabatic SLR refocusing MASE pulses for slice selection in the two other directions [6]. Briefly, a 5 ms SLR excitation pulse with 3.52 kHz bandwidth and a 2.5 ms adiabatic SLR refocusing pulse with 3.24 kHz bandwidth were used in the implementation of MASE. Two pairs of the same adiabatic SLR refocusing pulses with 2.5 ms duration and 3.24 KHz bandwidth were used for slice selection in the two other directions. The spoiler gradients have 25 mT/m amplitude and their duration varies between 0.8 to 2 ms. Four RF WET water suppression with less sensitivity to B1 variations than three RF WET [7] was used prior to the localization sequence.

### MEGA-sLASER (with MASE) with TE = 68 ms

For this study, we also implemented MEGA-sLASER (with MASE) sequence with TE = 68 ms at 7T as presented in[6]. For the localization part of the sequence, MASE pulses were also used. A combination of RF pulses and spoiler gradients as used in the implementation of MASE-sLASER described above was used here. Besides, a pair of dual band inversion pulses with bandwidth of 133 Hz and duration of 11.52 ms were used for MEGA editing and extra water suppression. MEGA-ON and MEGA-OFF modes are interleaved in the sequence as even and odd acquisitions, with MEGA pulse excitation centered at 1.9 ppm and 4.7 ppm in ON mode and 4.7 ppm and 7.5 ppm in OFF mode respectively. The same WET water suppression was used as above. The implementation for both techniques in terms of RF pulse profiles, voxel volume and water suppression was hence identical.

# Data acquisition

## In vivo

In total, 7 healthy subjects (2 female; age 29.7±5.8 years) participated in this study, with approval from the local ethics committee. In vivo scans were performed at 7T (Magnetom 7T, SIEMENS Healthcare GmbH, Erlangen, Germany) with 32 channel receiver and single channel transmitter head coil (Nova Medical, NY). An anatomical reference image was acquired using 3D MPRAGE [8] (384 slices, slice thickness = 0.5 mm, TR = 2500 ms, TE = 1.74 ms, TI = 1100 ms, Flip angle = 6°, FOV = 256 x 256 x 192 mm, 512 x 512 acquisition matrix, GRAPPA acceleration factor 2 PE, Ref. lines PE = 48, phase partial Fourier = 6/8, slice partial Fourier = 6/8, scan duration = 538 s). B0 shimming was performed using FASTESTMAP [9]. Single voxel MRS data were collected from two voxels of size 20x20x20 $mm^3$ positioned in a predominantly GM region and a predominantly WM region in the occipital lobe (figure 1) of 7 healthy subjects using MASE-sLASER sequence (TR = 4500 ms, TEs = 68 ms, NEX=64, scan time = 5:06 mins) and MEGA-sLASER sequence (TR = 4500 ms, TE = 68 ms, NEX = 64, scan time = 5:06 min).

# Data analysis

Data were analyzed using JMRUI [10] and MATLAB (version. 2016b, Natick, MA). Also, LCModel software (Version 6.3-1L, Stephen Provencher, Ontario, Canada) [11,12] was used to estimate metabolite concentrations. For the basis set for the LCModel analysis, parametric spectral models of alanine (Ala), aspartate (Asp), ascorbate (Asc), glycerophosphocholine (GPC), choline (Cho), phosphocholine (PCh), creatine (Cr), phosphocreatine (PCr), GABA, glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), GSH, myo-inositol (mI), lactate (Lac), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), scyllo-inositol (Scyllo) and taurine (Tau) were simulated using the NMRSIM module included in TOPSPIN suite (Version 3.6, Bruker, Rheinstetten, Germany). For each of the two acquisition sequences the basis set intensities were generated using TOPSPIN with identical parameters (e.g. RF pulse profile, resonance frequency and acquisition bandwidth) to those used for the in-vivo acquisition. Chemical shift and J-coupling values for each metabolite were taken from [13].

For the MASE-sLASER data, the basis set for the LCModel analysis consisted of twenty-one simulated metabolites. For the MEGA-sLASER editing data, six edited metabolites were modelled: GABA, Glu, Gln, NAA, NAAG, and GSH were included in the basis set. Each edited spectral model was created by subtracting a simulated MEGA-off spectrum from a simulated MEGA-on spectrum. As LCModel performs a phase correction during spectral fitting, additional phase correction steps were not applied. For MM and lipid signals, the non-parametric basis sets that LCModel provides by default were used [14]. We report GABA concentrations estimated by LCModel as a ratio relative to tNAA. We used the tNAA signal in the MEGA-off spectrum as an internal reference for the MEGA-editing approach. The NAA concentration was corrected for the MEGA spectra by multiplying it by a factor of two, because NAA signal is absent in MEGA ON mode. As unsuppressed water signal was not acquired in this study, it was not possible to estimate absolute concentration of the metabolites with LCModel.

GM and WM volume distributions within the spectroscopy voxels were calculated using T1-weighted images that were acquired as an anatomical reference before spectroscopy. We segmented the T1-weighted images of each spectroscopy voxel into GM, WM and CSF using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK) unified segmentation routines. The volume fraction for each tissue component was then calculated.

# Results

In table 1 we present the tissue compositions (relative GM and WM fractions) for each voxel together with the 3 ppm signal intensity of MEGA and 2.28 ppm signal intensity of MASE sLASER acquisitions.

| Table 1 | Subject 1 | | Subject 2 | | Subject 3 | | Subject 4 | | Subject 5 | | Subject 6 | | Subject 7 | | Mean±Std |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WM Voxel | GM Voxel | WM Voxel | GM Voxel | WM Voxel | GM Voxel | WM Voxel | GM Voxel | WM Voxel | GM Voxel | WM Voxel | GM Voxel | WM Voxel | GM Voxel | WM Voxel |
| GM/WM distribution | GM:22% WM:78% | GM:84% WM:16% | GM:12% WM:88% | GM:74% WM:26% | GM:12% WM:88% | GM:78% WM:22% | GM:26% WM:73% | GM:81% WM:17% | GM:21% WM:78% | GM:73% WM:23% | GM:27% WM:70% | GM:71% WM:14% | GM:12% WM:82% | GM:54% WM:20% | GM:18.8±6.7% WM:79.6±6.9% |
| $S_{3ppm(MEGA)}$ | 1509 | 1764 | 790 | 1259 | 1087 | 1258 | 1598 | 2045 | 981 | 1177 | 1230 | 1499 | 1724 | 1890 | 1274±346 |
| $S_{2.28ppm(MASE)}$ | - | 662 | - | 595 | - | 615 | - | 484 | - | 543 | - | 859 | - | 280 | - |

*Table 1: Tissue composition (GM and WM content), signal intensity at 3 ppm measured with MEGA-sLASER sequence and signal intensity at 2.28 ppm measured with MASE-sLASER sequence are shown for WM and GM voxels for all seven subjects.*

Figure 1. shows an example of MEGA-sLASER and MASE-sLASER spectra acquired at TE = 68 ms from predominantly GM and WM voxels of a healthy human brain (subject 3) and the voxel positions. The results from the other six subjects are provided in the supplementary materials (Supplementary figure 1). GABA line at 2.28 ppm in the MASE spectra from WM voxels was not clearly visible, most probably because of very low concentration of GABA in WM.

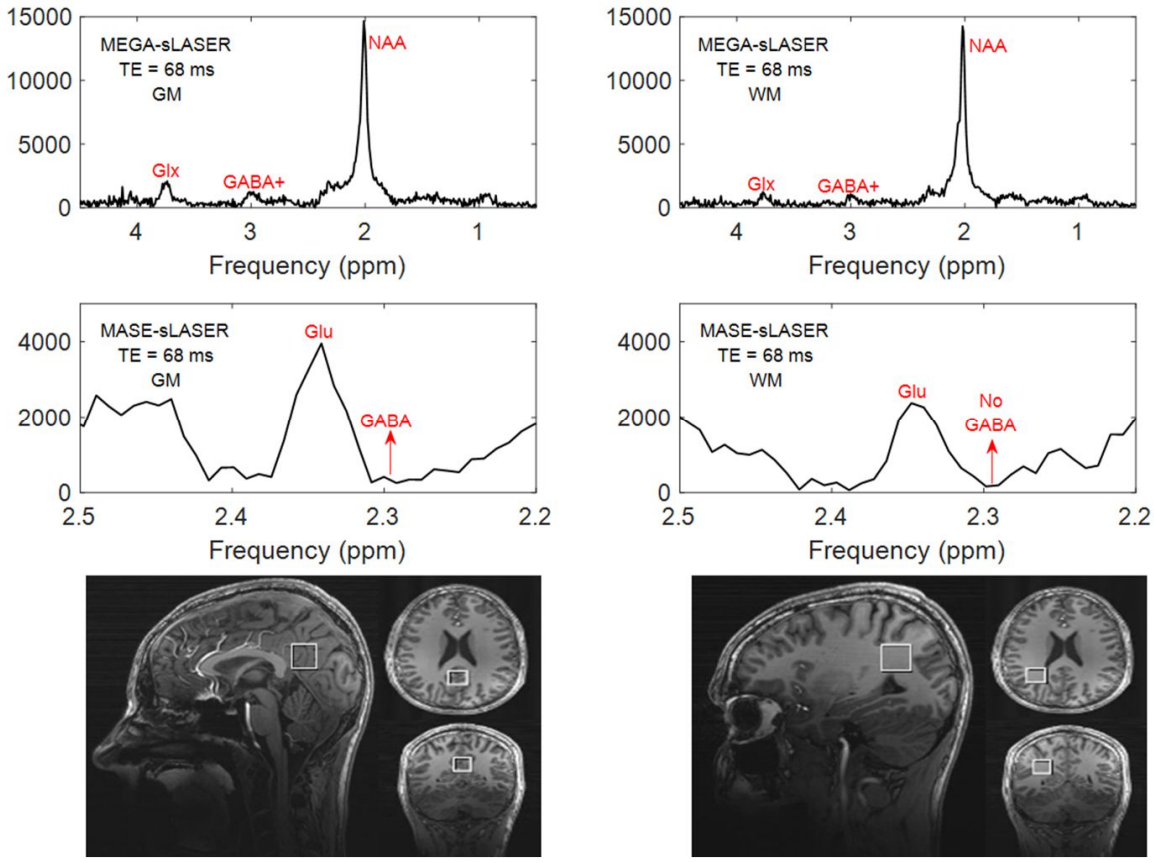



*Figure 1: MEGA-sLASER and MASE-sLASER spectra acquired at TE = 68 ms from predominantly GM (left) and WM (right) voxels positioned at the occipital region of a healthy human brain (subject 3).*

Table 2 summarizes GABA, Glu and NAAG ratios relative to tNAA estimated by LCModel for MEGA-sLASER and MASE-sLASER acquisitions in GM and WM voxels across seven subjects.

| Table 2 | MASE-sLASER, GM | | MEGA-sLASER, GM | | MASE-sLASER, WM | | MEGA-sLASER, WM | |
|---|---|---|---|---|---|---|---|---|
| Metabolite | met/NAA | CRLB% | met/NAA | CRLB% | met/NAA | CRLB% | met/NAA | CRLB% |
| | Subject 1 | | | | | | | |
| GABA | 0.107 | 54% | 0.148 | 12% | 0.08 | 76% | 0.099 | 18% |
| Glu | 1.003 | 8% | 0.935 | 6% | 0.737 | 11% | 0.666 | 9% |
| NAAG | 0.0075 | 117% | 0.041 | 29% | 0.057 | 16% | 0.106 | 14% |
| | Subject 2 | | | | | | | |
| GABA | 0.082 | 82% | 0.151 | 15% | 0.066 | 121% | 0.073 | 39% |
| Glu | 0.93 | 13% | 1.067 | 8% | 0.297 | 58% | 0.509 | 27% |
| NAAG | 0.017 | 73% | 0.063 | 27% | 0.064 | 25% | 0.118 | 24% |
| | Subject 3 | | | | | | | |
| GABA | 0.076 | 74% | 0.156 | 12% | 0.061 | 107% | 0.033 | 60% |
| Glu | 1.043 | 8% | 1.026 | 6% | 0.849 | 11% | 0.481 | 12% |
| NAAG | 0.00027 | 999% | 0.078 | 18% | 0.077 | 13% | 0.154 | 9% |
| | Subject 4 | | | | | | | |
| GABA | 0.078 | 82% | 0.141 | 15% | 0.11 | 57% | 0.102 | 18% |
| Glu | 0.919 | 12% | 1.035 | 7% | 0.644 | 14% | 0.674 | 10% |
| NAAG | 0.0072 | 155% | 0.088 | 18% | 0.017 | 57% | - | - |
| | Subject 5 | | | | | | | |
| GABA | 0.097 | 64% | 0.162 | 12% | 0.054 | 116% | 0.084 | 23% |
| Glu | 1.051 | 9% | 1.032 | 6% | 0.822 | 11% | 0.66 | 9% |
| NAAG | 0.01 | 98% | 0.062 | 22% | 0.076 | 11% | 0.106 | 13% |
| | Subject 6 | | | | | | | |
| GABA | 0.16 | 43% | 0.172 | 12% | 0.08 | 85% | 0.127 | 15% |
| Glu | 1.077 | 10% | 1.115 | 6% | 0.665 | 16% | 0.754 | 9% |
| NAAG | 0.011 | 107% | 0.078 | 20% | 0.018 | 61% | - | - |
| | Subject 7 | | | | | | | |
| GABA | 0.11 | 60% | 0.155 | 14% | 0.075 | 90% | 0.085 | 32% |
| Glu | 1.128 | 10% | 1.066 | 8% | 0.784 | 13% | 0.734 | 14% |
| NAAG | - | - | 0.052 | 34% | 0.043 | 23% | 0.132 | 18% |

*Table 2: LCModel estimates for GABA, Glutamate, and NAAG ratios relative to tNAA and their corresponding CRLBs for MASE-sLASER and MEGA-sLASER acquisitions in predominantly GM and WM voxels across seven subjects.*

Figure 2 (left) shows the correlation between GABA/tNAA of MEGA and GABA/tNAA of MASE acquisitions both estimated by LCModel. We found a linear correlation between these two ratios across all subjects and all voxels. The linear regression line was $(GABA/tNAA)_{MASE} = 0.9418 * (GABA/tNAA)_{MEGA} + 0.033$. The p value and standard deviation for the slope of the linear regression were 0.008 and 0.296 respectively. The p value and the standard deviation for the intercept were 0.086 and 0.0177 respectively. The $R^2$-value of this linear regression was 0.45.

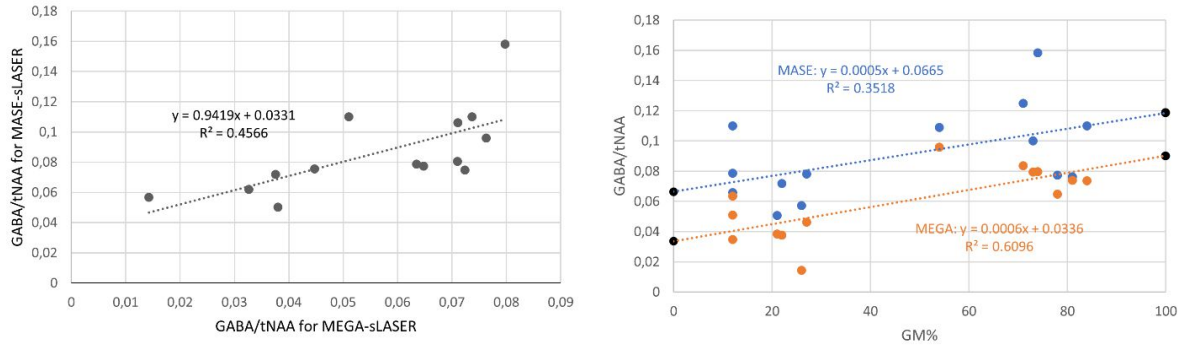



Figure 2: Left: best fit for the GABA/tNAA ratios estimated with LCModel for MASE-sLASER and MEGA-sLASER acquisitions at TE = 68 ms. The slope of the regressed line is almost one (0.94) confirming that the two techniques are measuring similar concentration of GABA when analyzed by LCModel. $R^2$ of the linear regression is also shown in the plot. Right: best fit of GABA/NAA ratio versus GM fraction for MASE-sLASER (blue) and MEGA-sLASER (orange). The fitted line and the corresponding $R^2$ are shown in the plots.

In figure 2 (right), we show the best fit and the corresponding $R^2$ of the GABA/tNAA ratio relative to GM fraction for MASE-sLASER (blue) and MEGA-sLASER (orange) acquisitions separately. The slope of the derived regression lines for the MASE and MEGA techniques are very close to each other, as to be expected from the slope of the left figure being near one. The intercepts of the two fitted lines at GM = 0% (WM = 100%) and GM = 100% are shown as black points in the graph. They give the predicted relative GABA ratio in pure GM and pure WM tissue for each acquisition method. The ratio of GABA in pure GM and pure WM calculated from the intercepts were 1.78 for MASE-sLASER method and 2.69 for MEGA-sLASER method.

# Discussion

It is well known from the literature that 3 ppm signal measured with MEGA editing technique is not fully GABA and contaminants (MM) contribute substantially to the edited 3 ppm signal. There are some strategies presented in the literature to measure the MEGA edited 3 ppm line free of contamination. Symmetric editing[15] and pre-inversion[3,16] are among them. Here we present a more straightforward approach basically relying on 2.28 ppm line which has hitherto received little attention. Moreover, in our acquisitions, although no individual peak was detected with MASE-sLASER at 2.28 ppm for WM voxels, there is a significant 3ppm signal in

the same voxel (WM) acquired with MEGA-sLASER (Table 1, Figure 1). This means that the absolute signal intensity at 2.28 ppm measured with MASE and at 3 ppm measured with MEGA do not represent exactly the same metabolites.

Our estimation of 3 ppm MEGA edited GABA with LCModel at 7T is however MM free. There are two arguments supporting this assertion. First, because the narrow bandwidth editing pulse (BW=133Hz) does not invert the 2.28 ppm line, the G ABA line at 2.28 ppm contributes in the MEGA edited spectra in the same fashion as 3 ppm GABA line but with the important feature of lacking MM contamination. Compared with 2.28 ppm line measured with MASE-sLASER, the 2.28 ppm line here is not separated from the neighboring Glutamate. LCModel however will still incorporate it to estimate the GABA signal. Second, the linear correlation between the GABA/tNAA ratio measured with the MEGA-sLASER sequence and GABA/tNAA ratio measured with MASE-sLASER sequence which are both estimated by LCModel has the slope of about 1 (0.94) (figure 2, left). This shows a very strong correlation between the two distinct methods to measure GABA when LCModel is used.

The GABA ratio in pure GM relative to in pure WM that is derived from intercepts of the best fits of MASE-sLASER and MEGA-sLASER (figure 2, right) represent however slightly different values (1.78 for MASE-sLASER and 2.69 for MEGA-sLASER). Both values lie well in the range of what has previously been reported in the literature[17-21]. Although, the GABA concentration in pure GM relative to in pure WM measured in different studies using different MRS acquisition methods reported in the literature are diverse with the range reported between 1.5 to 8[17-24]. The ratio of 1.78 derived from MASE-sLASER technique in this study matches with reports by Geramita et al[18], Mikkelsen et al[20], and Zhu et al[21]. The ratio of 2.69 derived with the MEGA-sLASER technique in this study on the other hand matches with reports by Choi et al[23] and Jensen et al[19].

# Conclusions

We conclude that LCModel analysis of MEGA spectra measured with MEGA-sLASER (with MASE) sequence at TE = 68 ms at 7T estimates MM free GABA concentration. We also validated the direct measurement of 2.28 ppm line with MASE-sLASER sequence by finding a linear correlation between LCModel analysis of the two distinct methods of MASE-sLASER and

MEGA-sLASER performed at the same TE = 68 ms. We also conclude that when LCModel is used, the 2.28 ppm line of GABA is much more crucial than the two other GABA lines to estimate MM free GABA concentrations with MEGA-sLASER and MASE-sLASER techniques at 7T.

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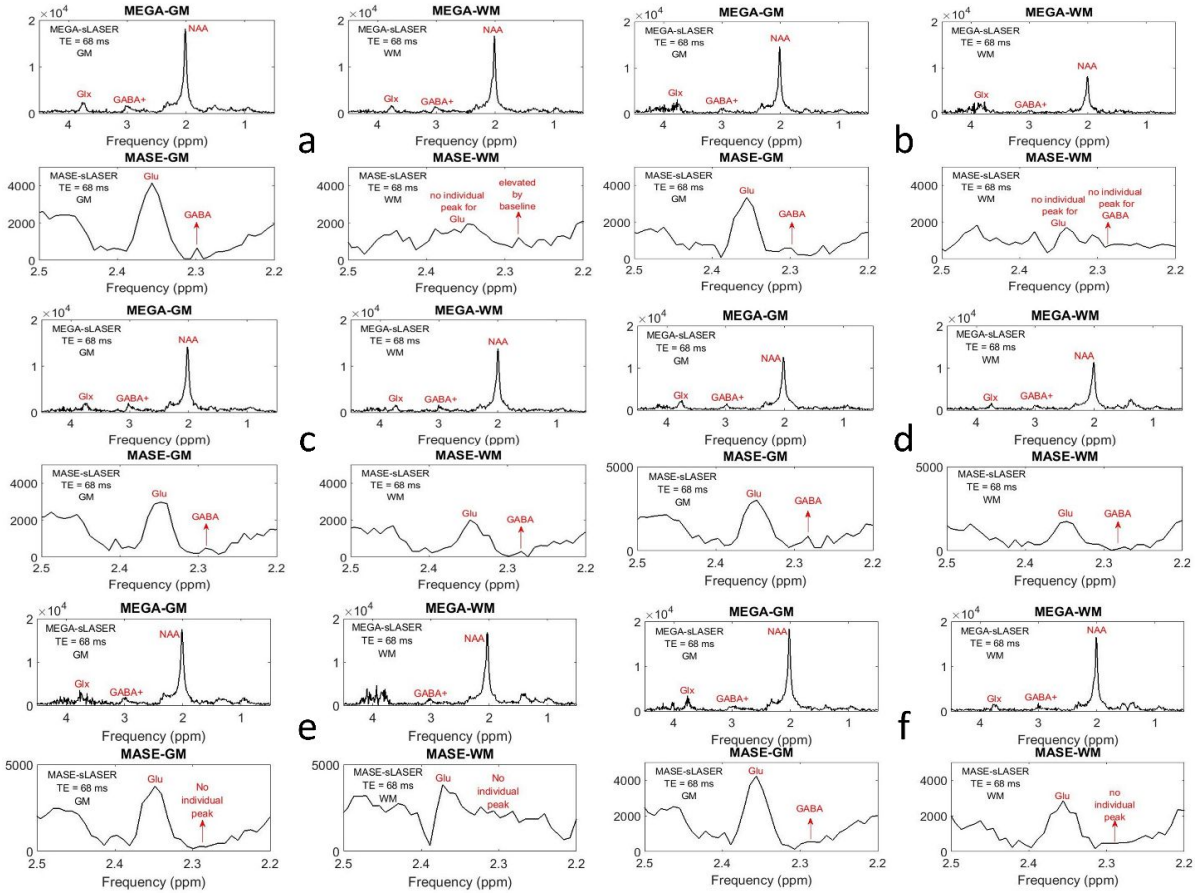
# Supplementary material




Supplementary figure 1: MEGA-sLASER and MASE-sLASER spectra acquired at TE = 68 ms from a GM voxel and a WM voxel positioned at the occipital region of healthy human brain (a: subject 1. b: subject 2, c: subject 4, d: subject 5. e: subject 6, f: subject 7).

# Chapter 5 - Antiphase J-difference editing method

Seyedmorteza Rohani Rankouhi, Donghyun Hong, David G. Norris

## Abstract

A J difference editing approach named 'antiphase J difference editing' method is implemented for proton single voxel spectroscopy. The technique brings the possibility of editing J coupled metabolites, here GABA, at any TE if the TE is long enough to satisfy the localization and editing timing conditions. The edited 3 ppm signal is observed at long TEs whereby the resonance at 1.89 ppm is inverted with a very narrow bandwidth pulse (36 Hz) which avoids inverting at 1.7 ppm. Considering the relatively short T2 of GABA at 7T reported in the literature (63-87ms), it is hypothesized that the edited signal at 3 ppm measured with this technique at long TEs in vivo originates from long T2 contaminants presumably in the form of mobile macromolecules. Indeed, in addition to the presence of macromolecule with coupling between 1.7 ppm and 3 ppm which is well known, we find evidence here for the presence of contaminants with long T2 and coupling between 1.89 ppm and 3 ppm, which would impact estimates of GABA concentration, especially at long TE.

## Introduction

Magnetic resonance spectroscopy (MRS) is currently the only noninvasive way to measure GABA in vivo [1,2]. Among the different MRS methods for measuring GABA, MEGA editing [3] is the most common. The method is based on the J evolution of GABA signals. This method is limited to certain TEs. The reason is that the side peaks of GABA at 3 ppm have to be naturally inverted by J evolution, which happens at TE=68 ms and at all higher odd-numbered multiples of this TE. Therefore, the MEGA editing method is an in-phase method.

There is an underlying spectrum covering a broad range of frequencies which constitutes the baseline, especially for short TE brain proton MRS. It is believed to mainly originate from proteins with high molecular weight and short T2 relaxation time (macromolecules (MM)). Behar et al have comprehensively investigated MMs in the rat brain [4] and the human brain [5].

It is well known that the J edited [6] or MEGA edited [3] 3 ppm signal can be severely contaminated by macromolecule (MM) contributions [6-9]. In the J editing or MEGA editing method, the GABA resonance at 1.89 ppm is inverted in the ON mode and is left unaffected in OFF mode at TE = 68 ms. Therefore, any metabolite that resonates at or close to 1.89 ppm and has a coupling partner at 3 ppm will be co-edited with GABA at 3 ppm. This is true if the coedited metabolite is within the bandwidth of the editing pulse. The contamination of the GABA 3 ppm signal measured with the J editing method had been hence the subject of a number of studies [7-11]. Rothman et al reported that the GABA J edited signal can consist of up to 60% macromolecule [6]. Shungu et al found 41-49% MM contribution in the J edited GABA+ signal in three cortical regions of the human brain [10].

Two different experimental approaches have been introduced in the literature to tackle the issue of MM contribution in the edited spectra. One method nulls the metabolite signals by applying an inversion pulse at an appropriate TI prior to the localization block [5,6]. This method is a general approach that can also be used in the specific case of MEGA editing. The other experimental technique for removing MM contributions, specifically for MEGA was introduced by Henry et al [9]. This experimental technique includes the inversion of resonances at 1.9 ppm and 1.5 ppm in the two modes of MEGA. The inversions in this technique are thus applied symmetrically about the J coupled MM resonance at 1.7 ppm and its contribution should be the same in the ON and OFF modes, of MEGA and thus cancel. However, the fixed TE of the MEGA technique represents a limitation to exploring macromolecular contamination of the 3 ppm MEGA edited signal, employing this symmetric inversion.

The assumption that all MM have a short T2 is not necessarily true. Choi et al [12] found evidence for a long T2 contaminant at 3ppm. Indeed, MMs in mobile form possess a long T2 relaxation time, and will contribute to the metabolic spectra even at long TEs. Using a double quantum filter with GABA-tuned and MM-tuned filters , the linewidth of GABA and MM lines

at 3 ppm reported by Choi et al [12] were found to be similar. Their finding provides evidence for the presence of macromolecular contaminants possessing long T2 relaxation times and contributing to the edited 3 ppm signal.

Here we introduce a J-difference editing technique named the antiphase J-difference editing method. The timing condition used in the design of the antiphase editing method presented here brings the benefit of preserving the editing capability at any TE provided that the TE is long enough to include all elements of the sequence. The method is therefore not like MEGA which is limited to fixed multiples of TE = 68 ms. The fixed TE = 68 ms for MEGA method hinders the possibility of using long editing pulses with narrow enough bandwidth to avoid inverting the contributing MM at 1.7 ppm. With the antiphase editing method however, we can use narrow bandwidth long editing pulse to avoid inverting the well-known 1.7 ppm MM and therefore we can use this approach to investigate the 3 ppm antiphase edited signal at long TE. The capability to use narrow bandwidth editing pulses also brings the possibility of editing both 2.28 ppm and 3 ppm signals simultaneously.

# Methods

## principle

The antiphase editing method we present here is a J-difference editing approach. The antiphase editing sequence consists of periodic repetitions of two modes, named here mode1 and mode2 that are interleaved in the sequence as odd and even acquisitions. If the time between excitation and editing pulses is T1 and that between editing pulse and acquisition is T2, then as depicted in Figure 1, mode 1 satisfies the timing condition $T_2 - T_1 = 33$ ms and mode 2 satisfies the timing condition $T_1 - T_2 = 33$ ms. Taking the average J coupling of GABA at 3 ppm to be 7.51 Hz [13], by having $T_1 - T_2$ or $T_2 - T_1 = 33$ ms = 1/4J, the two side peaks of GABA will be +90 and -90 degrees out of phase relative to the central peak. Consequently, subtracting the signal of the two modes will eliminate the central peak which is overlapped by non-coupled Creatine and reveals the two side peaks in antiphase. To avoid the two antiphase lines canceling out each other, the frequency difference between the lines must be greater than the linewidth.

## sequence implementation

The antiphase editing method was implemented in a sLASER sequence at 7T. The antiphase editing sequence has an editing pulse with duration of 42 ms and bandwidth of 36 Hz to invert at 1.89 ppm in one set of measurements and at 1.7 ppm in the other. The implemented sequence is shown in Figure 1. In the implementation of the localization part of the sequence which is sLASER [14,15], a 90° conventional SLR pulse and two pairs of 180° adiabatic SLR pulses were used. The 90° excitation pulse had a duration of 3.4 ms and bandwidth of 3.5 KHz, and the 180° adiabatic SLR refocusing pulses had duration of 2.5 ms and a bandwidth of 3.24 KHz. A 4 RF WET water suppression block was used before the localization block of the sequence [16]. There are two sets of timing conditions in the design of the antiphase editing sequence, a general timing condition to complete the single voxel localization with the sLASER sequence and the editing timing condition depicted in Figure 1. Using very long editing pulse with the duration of 42 ms together with the localization RF pulses and spoiler gradients and the two governing timing conditions gave a minimum TE of 195 ms for the antiphase editing sequence at 7T.

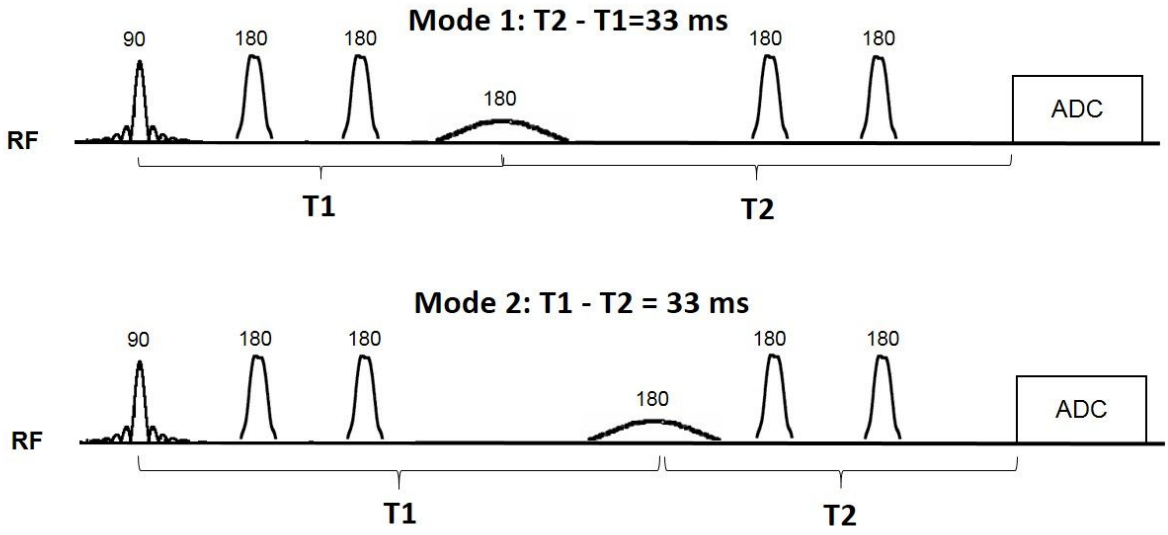



*Figure 1: Antiphase J-difference editing sequence in two modes, mode1 and mode 2 with their governing timing conditions implemented in a sLASER sequence at 7T.*

# Simulation

NMR-SIM (TopSpin Version 3.7, Bruker Biospin, Germany) was used to simulate GABA signals for modes 1 and 2. Identical acquisition sequence and parameters such as RF pulse profile, BW and resonance frequency were used. Chemical shift, J-coupling and T2 information for GABA were obtained from reported literature values [13,17]. More specifically GABA T2 of 63 ms was used for the simulation [17]. A difference spectrum at each TE was generated by subtracting individually simulated mode 1 and 2 spectra in the frequency domain.

# Data acquisition

## Phantom

Spectra were acquired from a phantom containing 30 mMol GABA in water at TEs from 200 ms to 900 ms incremented in 50 ms steps using the antiphase editing sequence (TR=4500 ms, NEX=32, scan time = 2:33 mins) from a voxel of size 30x30x30 $mm^3$ using a 7T system (Magnetom 7T, SIEMENS Healthcare GmbH, Erlangen, Germany) with 32 channel receiver and single channel transmitter head coil (Nova Medical, NY). These phantom measurements were used to verify the capability of the antiphase J difference editing method to edit GABA resonances at both 3 ppm and 2.28 ppm. We also performed simulations with the same spectroscopy parameters to evaluate the performance of the editing sequence to edit 3 ppm and 2.28 ppm GABA signals.

## In vivo

We performed two sets of measurements to investigate the antiphase edited 3 ppm signal. In one set of measurements we applied the narrow bandwidth editing pulse at 1.7 ppm. In another set of measurements, we applied the narrow bandwidth editing pulse at 1.9 ppm.

In total, 4 healthy subjects (2 female; age 27.2±4.6 years) participated in this study with approval from the local ethics committee. An anatomical reference image was acquired using 3D MPRAGE [18] (256 slices, slice thickness = 1 mm, TR = 2500 ms, TE = 1.35 ms, TI = 1100 ms, Flip angle = 6°, FOV = 256 x 256 x 256 mm, 256 x 256 acquisition matrix, GRAPPA acceleration factor 2 PE, Ref. lines PE = 48, phase partial Fourier = 6/8, slice partial Fourier = 6/8, scan duration = 298 s). B0 shimming was performed using FASTESTMAP [19]. Spectra were acquired

from a 30x30x30 $mm^3$ voxel placed in the medial occipital region using the antiphase editing sequence at TEs 195, 225 and 255 ms (TR=4500 ms, NEX=64, scan time = 5:06 mins). Data were analyzed using JMRUI [20] and MATLAB (version. 2016b, Natick, MA). The relatively large voxel size was selected to compensate for the low concentration of GABA and the relatively long TEs used for in vivo measurements.

When the bandwidth of the editing pulse is very narrow there is the danger of sensitivity to frequency drift. To avoid this, the carrier frequency was adjusted prior to each individual spectroscopy acquisition.

# Results

## Simulation and Phantom

Figure 2 demonstrates simulation results showing the edited GABA signal and its oscillating decay with increasing TE for both 2.28 and 3 ppm resonances.

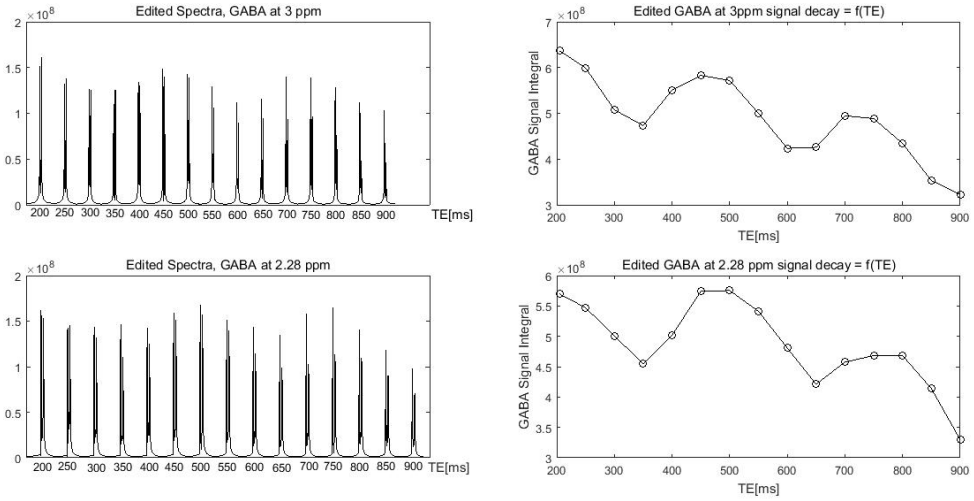



*Figure 2: Simulation results for the antiphase J difference editing sequence showing the edited spectra of 2.28 ppm and 3 ppm GABA resonances at TEs 200 ms to 900 ms and the oscillating decay of the edited signals with increasing TE for these two resonances.*

In figure 3, we show the edited 2.28 ppm and 3 ppm signals acquired from phantom presenting an oscillating decay of the edited GABA signals with increasing TE. Apart from oscillation, the two GABA resonances at 3 ppm and 2.28 ppm demonstrate similar T2 decays in phantom results.

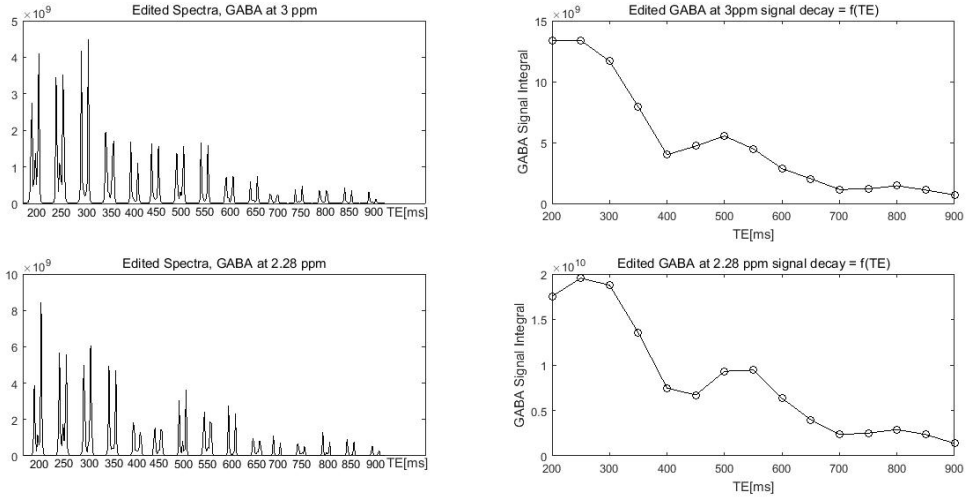



*Figure 3: phantom results for the antiphase J difference editing sequence showing the edited spectra of 2.28 ppm and 3 ppm GABA resonances at TEs 200 ms to 900 ms and the oscillating decay of the edited signals with increasing TE for these two resonances.*

The absence of the central peak in the edited spectra confirms successful editing of both GABA resonances at 2.28 ppm and 3 ppm using the antiphase J difference editing approach. There is a good match between simulation and phantom results. The origin of the signal oscillation will be discussed below.

## In vivo

Antiphase edited spectra acquired at TEs 195, 225 and 255 ms from a 30x30x30 $mm^3$ voxel positioned in the medial occipital region of four healthy subjects when inverting at 1.89 ppm in one set of measurements and at 1.7 ppm in another set of measurements are shown in Figure 4. Applying the inversion pulse at 1.89 ppm and at 1.7 ppm in two separate sets of measurements both led to an edited signal at 3 ppm with no coedited signal at 2.28 ppm. There is also a 2 ppm coedited signal in the edited spectra.

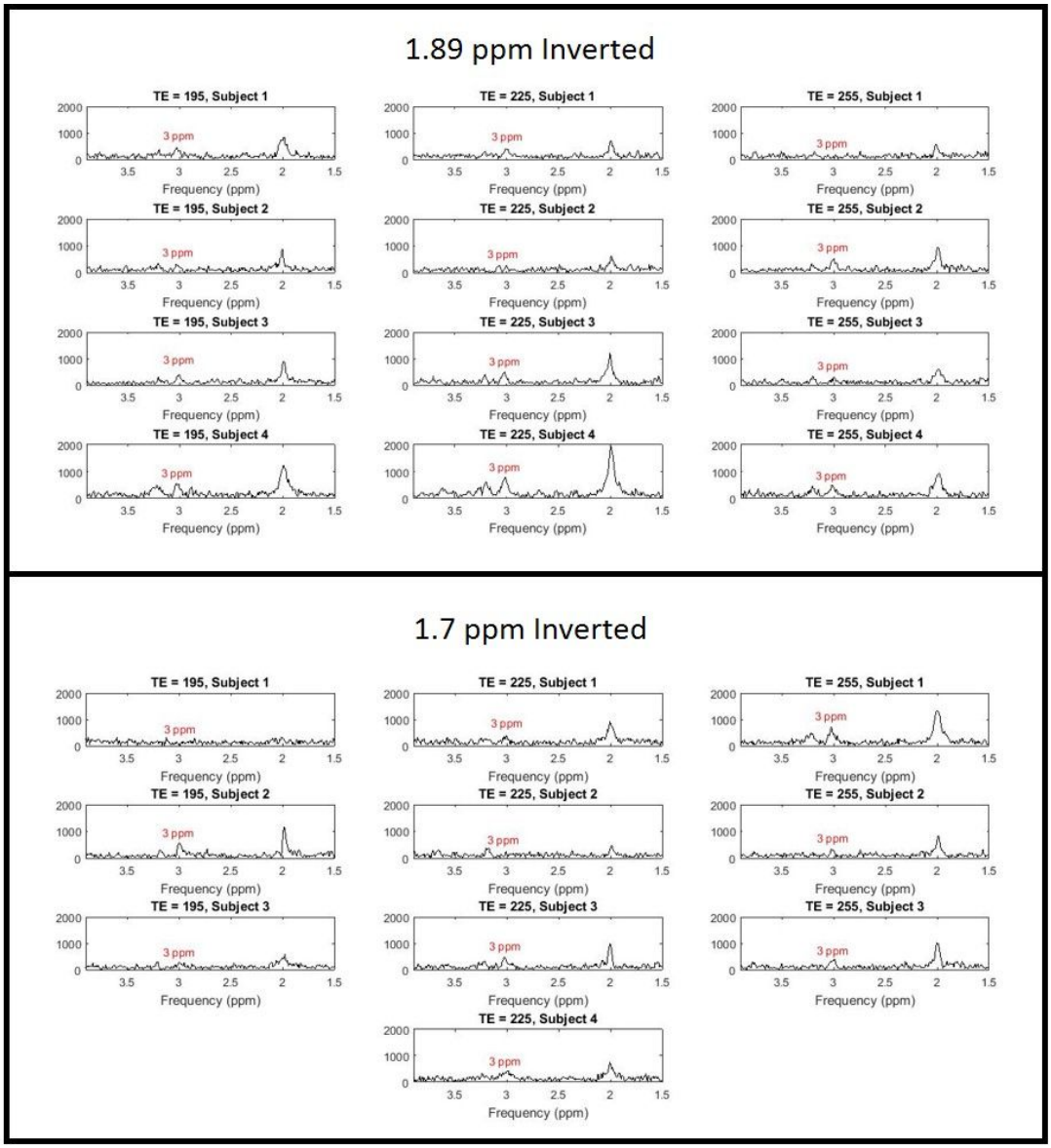



*Figure 4: Antiphase edited spectra acquired at three TEs of 195, 225 and 255 m for subjects 1 to 4 measured in a 30x30x30 $mm^3$ voxel placed in the medial occipital region. The editing pulse was applied at 1.89 ppm (above) and 1.7 ppm(bottom). Edited 3 ppm signal is labeled in the spectra. Note that there is no coedited 2.28 ppm signal in the edited spectra. Also, a 2 ppm coedited signal is visible in all spectra. spectra at TEs 195 ms and 255 ms for the subject 4 are not shown for inversion at 1.7ppm because of a high difference artifact making the signals artificially very high.*

# Discussion

While phantom and simulation results both confirm the successful editing of the 3 ppm and 2.28 ppm GABA signals with the antiphase editing method, as demonstrated by having the central peak of GABA absent in the edited spectra, there remains an oscillation in the signal intensity of the edited spectra of both 3 ppm and 2.28 ppm GABA resonances as a function of

TE. The antiphase editing method would make the edited signal intensity independent of TE if all coupling constants were exactly equal, and we should then observe an exponential signal decay. This corresponds to the assumption that 2.28 ppm and 3 ppm are perfect triplets with the two side peaks equidistant from the central peak. In reality, this is not the case, and the J coupling constants for the protons of each resonance are unequal [13]. We hypothesise that the unequal coupling constants of the protons of the GABA lines at 2.28 pm and 3 ppm are the reason for the oscillation as a function of TE.

## antiphase edited signal at 3 ppm

In the antiphase editing sequence, we assign the 3 ppm edited signal measured by inverting at 1.7 ppm to a contaminant, presumably mobile MM with a long T2 relaxation time coupled to 1.7 ppm as investigated previously [4,5]. This is consistent with and supports the finding of Choi et al [12].

Furthermore, in another set of measurements we inverted 1.89 ppm with a 36 Hz bandwidth editing pulse. Therefore, the well-known coupled MM signal at 1.7 ppm is not inverted, and its coupling partner will not contribute to the 3 ppm edited signal. A feature of the in vivo antiphase edited spectra in this set of measurements is however the absence of the GABA peak at 2.28 ppm. If the observed signal at 3 ppm in this set of measurements has a significant contribution of GABA, then coedited GABA at 2.28 ppm should be visible with a similar intensity. This is because the editing pulse is so narrow that it does not invert the coupled GABA peak at 2.28 ppm. This argument is based on the assumption that the T2s of the GABA resonances at 3 ppm and 2.28 ppm are similar, which was the case in our phantom measurements. It is unlikely that the T2s of these resonances would then differ for a small mobile molecule like GABA in vivo. Thus, the absence of a 2.28 ppm signal suggests that the measured 3 ppm signal is not GABA. In fact, the result when applying the editing pulse at 1.89 ppm presented here suggests the hypothesis of a contaminant at 1.89 ppm in vivo coupled to a partner at 3 ppm which was not investigated by Behar et al [4,5]. The 2D COSY spectrum presented by Behar et al in human brain [5] to investigate MMs was obtained from an extensively dialyzed brain tissue. Therefore, contaminants in mobile form would have been removed.

There are two T2 values reported in the literature for GABA at 7T in vivo, one 63 ms [17] and the other 87 ms [21]. Our in vivo acquisitions in the current study are at TEs 195, 225 and 255 ms. Considering T2 of GABA being 63 ms [17] or 87 ms [21] in vivo, the TEs used here are long and the GABA signal will substantially decay due to T2 relaxation effect, which further supports the hypothesis that the measured in vivo 3 ppm signal is not GABA.

Choi et al used a double quantum filter to investigate the 3 ppm edited signal [12]. They reported the presence of a contaminant other than MM presumably a mobile macromolecule with long T2 of 180 ms at 3T which contributes to the 3 ppm edited signal [12]. They estimated the concentration of GABA and long T2 contaminant in WM and GM voxels. However, it is likely that both signals measured in that study in WM are mainly contaminants: one originating from a coupling between 1.7 and 3 ppm and the other from a coupling between 1.89 and 3 ppm. There are some supportive facts for this hypothesis. Firstly, the ratio of GABA concentration in GM to WM reported by Choi et al is around 3 which is contrary to histological investigations which report a ratio of around 10 [22]. Moreover, Choi et al measured the 3 ppm signal at TE = 148 ms which is short enough to measure some GABA signal, even if T2 of GABA is assumed to be as short as 63 ms [17] or 87 ms [21]. Therefore, there would be some residual GABA signal at this TE of 148 ms in the GM voxel in that study. This can explain the different concentrations of the GABA + contaminant in GM and WM measured by Choi et al, even if working on the assumption that the concentrations of MM in GM and WM are similar [23]. Furthermore, assuming that the concentrations presented in table 1 of Choi et al [12] for the two components in WM originate from two groups of contaminants and not from GABA and contaminant, then one of the two groups of contaminants in WM can be designated as mobile MM with a coupling between 1.7 ppm and 3 ppm given a concentration of 0.7 mM. The other group of contaminants would then be mobile MM with coupling between 1.89 ppm and 3 ppm designated in that table as GABA with a concentration of 0.4 mM. This gives altogether total of 1.1 mM contaminant in WM. Then, if we assume similar concentration of MM in WM and GM [23], the signal from contaminant in GM in table 1 of Choi et al must be also 1.1 mM which leaves 0.8 mM GABA concentration in GM. The ratio of GABA to MM in GM with this calculation is then 42% and the rest (58%) is assigned to be MM. These calculated ratios (42%:58% for GABA:MM) agree with those previously reported in the literature [6,10]. The Choi

et al [12] results discussed above support our conclusion in the current study that at long TEs, such as used here, and with the antiphase J difference editing technique, we are measuring macromolecules at 3 ppm with long T2 relaxation time and coupling partners at 1.7 or 1.89 ppm. These are most probably MMs in mobile form and are present in vivo but cannot show up in the extensively dialyzed tissue samples used by Behar et al. [4,5].

## coedited signal at 2 ppm

Behar et al also investigated MMs in rat brain [4] where they tentatively assigned certain amino acids to the investigated MM peaks. Importantly, in that study not all the cross peaks could be assigned. The MM spectrum is very broad. Behar et al assigned the MM peaks in rat brain to seven major peaks (M1-M7) while a few minor peaks were also observed but not assigned. Interestingly, there were cross peaks at 2.03 ppm and 1.89 ppm in the 2D COSY spectrum which Behar et al tentatively assigned to Glu and Gln in their rat brain study [4]. In one of our two sets of antiphase measurements, where we inverted 1.89 ppm, we observed a coedited peak at 2 ppm. Our observation of a coedited 2 ppm signal is consistent with the finding of Behar et al. Based on their tentative assignment, we also assign this coedited signal to Glu and Gln. Furthermore, this peak cannot be residual NAA because the editing pulse was too narrow to invert it.

In another set of measurements where we inverted 1.7 ppm, there is also coedited signal at 2 ppm in the edited spectra. Pogliani et al [24] presented proline's chemical shifts and J couplings at different PHs. At PH = 7 which is close to in vivo conditions, proline has resonances at 1.6, 1.63, 1.68 and 1.96 ppm all coupled to each other [24]. For instance, there is -13.5 Hz coupling between proline at 1.68 ppm and proline at 1.96 ppm. The signal observed at 2 ppm in the antiphase edited spectra in this set of measurements is therefore assigned tentatively to proline. Again, the coedited signal in this set of measurements can not be residual NAA because the editing pulse was very narrow and was inverting 1.7 ppm much far away from 2 ppm.

# Conclusion

Our results indicate that MEGA edited spectra will always suffer from a coedited contaminant, even if the experimental approach proposed by Henry et al [9] is employed. The approach introduced by Henry et al eliminates the presence of coedited MM at 1.7 ppm but naturally cannot eradicate any coedited 1.89 ppm contaminant. Therefore, metabolite nulling together with fitting methods may be a more reliable approach to estimate the macromolecule free GABA concentration when measured with MEGA. This is true if we assume that the contaminants possess similar T1s which will be shorter than the metabolite T1. T1 of MM in the Choi et al publication was estimated to be about 450 ms and much shorter than that of metabolites [12]. The presence of contaminants with long T2 relaxation time must be considered when quantifying GABA concentrations or measuring the T2 of the 3ppm GABA resonance.

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# Chapter 6 - Summary

## Key features

- A new SVS acquisition method (interleaved sequence) has been introduced which can be used to compare two or more acquisition techniques by minimizing destructive factors emerging from separate acquisitions like B1 inhomogeneity, frequency drift and motion artefacts.
- a new version of sLASER sequence named MASE-sLASER sequence is introduced with the features of short TE, matched CSDE, possibility of direct measurement of GABA at 2.28 ppm at certain TEs and an application of implementing MEGA-sLASER with TE = 68 ms at 7T that are presented as this sequence's primary features and applications.
- The two distinct GABA measurement methods namely MASE-sLASER and MEGA-sLASER operating at the same TE = 68 ms are validated against each other. Macromolecule (MM) free GABA measurement with these two techniques was feasible with the aid of LCModel and of the detection of the 2.28 GABA line in the acquired spectra.
- a new editing technique named antiphase J difference editing method is introduced which can edit J coupled metabolites over a continuous range of TEs.
- It is found that there are long T2 contaminants that coedit in the antiphase J editing technique of 3 ppm GABA at long TEs.

## General discussion

J editing or MEGA editing method is currently among the most common MRS approaches to measure GABA. However, in terms of using the MEGA method at 7T, there are some limitations at ultrahigh field that need to be considered. First of all, B1 inhomogeneity increases at ultrahigh field. Therefore, in terms of localization, sLASER is much more reliable method than PRESS at ultrahigh field strengths because of the use of adiabatic pulses that are immune to B1 variations. Then comes the pitfall of the higher number of RF pulses in sLASER which impacts on minimum achievable TE and is thus a limitation. Secondly, in terms of MEGA editing, the bandwidth of the two editing pulses used in the sequence is very important. In

particular, it is known that there is MM at 3 ppm which is coupled to a coupling partner at 1.7 ppm. To exclude this MM from being coedited in the edited 3 ppm signal, it is important to have narrow enough editing pulses in the sequence. However, on the other side MEGA method is limited to certain TEs with most optimum one in terms of giving the highest SNR being 68 ms. Therefore, the timing of the method brings a limitation in the maximum possible duration and thus minimum possible bandwidth of the editing pulses. For this reason, at 3T for instance, TE was increased to 80 ms to make it possible to use narrow enough editing pulses. On the other hand, another possibility to measure GABA at ultrahigh field is to use standard sLASER. This localization technique is beneficial compared with other possible methods like PRESS, STEAM and SPECIAL because of its higher immunity to the B1 variations due to employing adiabatic pulses. To measure GABA with this technique however, fitting methods like LCModel are needed. The TE is shorter than for the MEGA method, which is a benefit, but GABA will be estimated using the fitting method from a larger number of different metabolites. There will be also a larger contribution of short T2 MMs presenting themselves as a broad baseline in the standard sLASER spectrum acquired at short to intermediate TE. In this thesis we developed techniques concerning the issues mentioned above and we also introduced new techniques especially for GABA measurement.

In **chapter 1** of this thesis, we summarized some key concepts that are needed to have a good understanding of the rest of the thesis. We summarized the main SVS localization techniques, water suppression techniques, also concepts like J coupling, chemical shift displacement error, as well as MEGA editing technique to measure GABA. In **chapter 2** our aim was to compare the two well established techniques exclusively for measuring GABA at ultrahigh field namely standard sLASER and editing MEGA-sLASER. We presented there an interleaved acquisition technique which should minimize the destructive factors when the acquisitions are performed separately, like B0 inhomogeneities, motion effects and frequency drift. The proton spectrum of GABA consists of three peaks at 1.89, 2.28 and 3.01 ppm. The lines at 2.28 ppm and 3 ppm are coupled to that at 1.89 ppm. J coupling, low concentration and overlap of the peaks with more concentrated other metabolites makes it very challenging and difficult to adequately measure GABA using MRS. However, while the two peaks of GABA at 1.89 ppm and 3 ppm are overlapped dominantly with other metabolites, 2.28 ppm is not exactly overlapped, and its

closest neighboring metabolite is Glutamate at 2.35 ppm. As ultrahigh field benefits from greater separation of chemical shifts due to increase in spectral resolution, the role of 2.28 ppm GABA at ultrahigh field emerges and its measurement at ultrahigh field has been of interest. In chapter 2 we also showed that this GABA resonance indeed plays a crucial role to estimate adequate overall GABA when LCModel fitting method is used. LCModel finds a proper fit for each metabolite by minimizing the fitting error and all GABA peaks are considered in the fitting process. Therefore, if the 2.28 ppm line estimated by LCModel is pure GABA, it helps adequate and MM free estimate of 3 ppm line in the MEGA edited spectrum. In **chapter 3** we introduced a new and extended version of sLASER named MASE-sLASER and presented some of its primary applications. First of all, we presented this sequence with TE as short as 27 ms which is comparable or lower than that of standard sLASER presented in the literature so far. The MASE-sLASER sequence additionally benefits from the feature of matched CSDE in all three directions which until now had been a feature of full LASER sequence and not of standard sLASER sequence. An improvement in terms of sequence components here are MASE pulses which are semi-adiabatic (excitation) and adiabatic (refocusing). This brings lower sensitivity to B1 inhomogeneity compared with the standard sLASER sequence which has a conventional non-adiabatic excitation pulse. MASE pulses also can operate at shorter duration compared with the hyperbolic secant pulses while preserving a reasonable bandwidth. This brought the opportunity to implement the sequence at short TEs despite having an extra RF pulse compared with the standard sLASER sequence. The shorter duration of MASE pulses also made it possible to implement the MEGA-sLASER sequence with TE = 68 ms at 7T. This can be compared with MEGA-sLASER that was implemented in chapter 2 using hyperbolic secant pulses. As another application of the MASE-sLASER sequence we showed the feasibility of measuring GABA at 2.28 ppm well separated from its neighboring Glutamate with this sequence at TE = 68 ms at 7T. GABA at 2.28 ppm is not overlapped with any dominant metabolite especially when the spectrum is acquired at higher fields where higher spectral resolution in beneficial. Measuring this GABA resonance directly without editing is of interest and a previous work by Gnaji et al presented this possibility using a long TE PRESS sequence at 7T. Two applications of MASE pulses presented in chapter 3 provide two distinct SVS acquisition methods for measuring two different GABA

resonances at the same TE. An application of this feature initiated the work we presented in **chapter 4**. With MASE-sLASER at TE = 68 ms we presented in chapter 3 the possibility of measuring GABA at 2.28 ppm well separated from its neighboring Glutamate. At the same TE with MASE pulses we implemented MEGA-sLASER sequence. By exploiting the detectability of the 2.28 GABA line in the spectra acquired with these two distinct techniques, we showed the feasibility of MM free GABA measurement and also validated the two techniques against each other. In **Chapter 5** we introduced the new antiphase J difference editing method. The idea of this technique is to preserve the side peaks of the GABA triplet in antiphase states between the two modes. By subtracting the two mode spectra, the central peak and the overlapping Creatine singlet will be eliminated, and the two side peaks are revealed in the antiphase state. In theory this technique works when the GABA peaks are symmetric with exactly equal coupling constants which is not the case in practice. Therefore, we observed the oscillation of the signal both in simulation and phantom results for long TE experiments. Otherwise, the technique must provide a mono exponential decay of the edited signal as a factor of TE. The observation of the oscillation of the antiphase edited signal both in simulation and in phantom measurements was not expected according the theoretical perspective assuming identical coupling constants. We explored further by applying this new editing technique to measure the 3 ppm signal in vivo. Because of timing constraints governing the sequence and the editing principal the minimum achievable TE was very long. The results however opened another window in terms of contamination of GABA J edited signal with MMs. We demonstrated in this chapter the presence of long T2 contaminants visible even at very long TEs. This signal should originate from mobile macromolecules that possess long T2s because of their mobility. An evidence for our conclusion in this chapter was the absence of the coedited 2.28 ppm GABA signal in the edited spectra when 1.89 ppm was inverted with a very narrow editing pulse, suggesting that the edited 3 ppm signal was not GABA.

## Open questions and future directions

A future work concerning MASE-sLASER technique is to investigate if there is any TE shorter than 68 ms at which this sequence can measure GABA at 2.28 ppm well separated from the neighboring Glutamate at 7T. A limitation in terms of measuring GABA at 2.28 ppm with the MASE-sLASER is the low numbers of TEs which we investigated in this thesis, being TEs 68 and

136 ms. Different patterns of Glutamate and GABA resonances in this region represent a potential limitation, because of overlap of these two signals, which makes it impossible to separate GABA and Glutamate at all TEs with the MASE-sLASER sequence. Also, the limited number of possible TEs in the MASE-sLASER method to measure GABA at 2.28 ppm makes it difficult to measure T2 of this GABA resonance with this technique. In fact, T2 relaxation time of the two GABA resonances are assumed to be similar to make it possible to compare the two resonances signal intensities without T2 correction. There are currently two studies in the literature that have estimated T2 of GABA (3 ppm edited) to be 63 ms and 87 ms. However, there is currently no report for the T2 of 2.28 ppm GABA in the literature. At present we have only been able to measure the 2.28 ppm signal at 68 and 136ms, but a full T2 measurement would require multiple TEs and to take the J-coupling of this resonance into account.

The contaminants which we found with long T2 at 3 ppm can be the subject of further research. One possibility would be to investigate their relative concentration in white matter (WM) and gray matter (GM) tissue in the brain. There is some evidence in the literature that MMs have similar concentrations in GM and WM. The investigated contaminant at 3 ppm can also be subject of research to find whether it can act as a marker for pathogenesis. MMs in general have been shown to be markers for several diseases including multiple sclerosis (MS) or for tumor in the brain. Measuring the T2 of these contaminants can also be a goal for future research, though may be their complicated J coupling could be a limitation in this context.

The antiphase editing sequence can be implemented in other localization techniques too. The editing timing condition of this method brings the opportunity to implement the method possibly at shorter TE than the 68 ms as of MEGA, and therefore to benefit from higher SNR. In this context implementing the antiphase editing method in the SPECIAL sequence for instance, could be the subject of future work. The antiphase editing method can also be used for editing other J coupled metabolites.

# Chapter 7 - Samenvatting

## Belangrijkste kenmerken

- Een nieuwe SVS-acquisitiemethode (interleaved sequence) is geïntroduceerd die kan worden gebruikt om twee of meer acquisitietechnieken te vergelijken door het minimaliseren van destructieve factoren die gepaard gaan met afzonderlijke acquisities zoals B1 inhomogeniteit, frequentieverschuiving en bewegingsartefacten.
- een nieuwe versie van de sLASER-sequentie met de naam MASE-sLASER-sequentie wordt geïntroduceerd met de kenmerken van korte TE, gematchte CSDE, de mogelijkheid van directe meting van GABA bij 2,28 ppm bij bepaalde TE's en een toepassing voor het implementeren van MEGA-sLASER met TE = 68 ms bij 7T, die worden gepresenteerd als de primaire kenmerken en toepassingen van deze sequentie.
- De twee verschillende GABA-meetmethoden, namelijk MASE-sLASER en MEGA-sLASER die op dezelfde TE = 68 ms werken, worden onderling gevalideerd. Macromolecule (MM) vrije GABA-meting met deze twee technieken was mogelijk met behulp van LCModel en van de detectie van de 2,28 GABA-lijn in de verkregen spectra.
- een nieuwe bewerkingstechniek op basis van verschillen met de naam antiphase J wordt geïntroduceerd die J-coupled metabolieten over een continu bereik van TE's kan bewerken.
- Het blijkt dat er lange T2-contaminanten zijn die gemeenschappelijk bewerken in de antiphase J-bewerkingstechniek van 3 ppm GABA bij lange TE's.

## Algemene discussie

J-bewerking of MEGA-bewerkingsmethode is momenteel een van de meest gebruikte MRS-methoden om GABA te meten. Wat het gebruik van de MEGA-methode bij 7T betreft, zijn er echter enkele te overwegen beperkingen op het ultrahoge veld. Op de eerste plaats neemt de B1-inhomogeniteit toe in het ultrahoge veld. In termen van lokalisatie is sLASER daarom een veel betrouwbaardere methode dan PRESS bij ultrahoge veldsterktes vanwege het gebruik van adiabatic pulses die immuun zijn voor B1-variaties. Dan komt de valkuil van het hogere

aantal RF-pulsen in sLASER die van invloed is op de minimaal haalbare TE en dus een beperking vormt. Op de tweede plaats, in termen van MEGA-bewerking, is de bandbreedte zeer belangrijk van de twee bewerkingspulsen die in de sequentie worden gebruikt. Het is met name bekend dat er een MM is met 3 ppm, die gekoppeld is aan een koppelingspartner met 1,7 ppm. Om uit te sluiten dat deze MM in het bewerkte 3 ppm-signaal gemeenschappelijk wordt bewerkt, is het belangrijk om voldoende smalle bewerkingspulsen in de reeks te hebben. Aan de andere kant is de MEGA-methode echter beperkt tot bepaalde TE's, waarbij de meest optimale de hoogste SNR krijgt van 68 ms. Daarom brengt de timing van de methode een beperking met zich mee van de maximaal mogelijke duur en daarmee de minimaal mogelijke bandbreedte van de bewerkingspulsen. Om deze reden werd bijvoorbeeld bij 3T de TE verhoogd naar 80 ms om het mogelijk te maken om voldoende smalle bewerkingspulsen te gebruiken. Een andere mogelijkheid om GABA te meten in het ultrahoge veld is het gebruik van een standaard sLASER. Deze lokalisatietechniek is gunstig in vergelijking met andere mogelijke methoden zoals PRESS, STEAM en SPECIAL vanwege de hogere immuniteit voor de B1-variaties door het inzetten van adiabatic pulses. Om GABA met deze techniek te kunnen meten zijn er echter aanpassingsmethoden zoals LCModel noodzakelijk. De TE is korter dan bij de MEGA-methode, wat een voordeel is, maar GABA zal worden geschat via de aanpassingsmethode vanuit een groter aantal verschillende metabolieten. Er zal ook een grotere bijdrage zijn van korte T2-MM's die zich presenteren als een brede baseline in het standaard sLASER-spectrum dat op korte tot tussenliggende TE wordt verkregen. In dit proefschrift hebben we technieken ontwikkeld met betrekking tot de hierboven genoemde onderwerpen en hebben we ook nieuwe technieken geïntroduceerd, met name voor GABA-metingen.

In **hoofdstuk 1** van dit proefschrift hebben we een aantal sleutelbegrippen samengevat die nodig zijn om de rest van het proefschrift goed te kunnen begrijpen. We geven een samenvatting van de belangrijkste SVS-lokalisatietechnieken, wateronderdrukkingstechnieken, ook van concepten als J-coupling, chemische verschuivingsfout en de MEGA-bewerkingstechniek voor GABA-meting. In **hoofdstuk 2** was het doel de twee beproefde technieken te vergelijken die uitsluitend voor GABA-meting in het ultrahoge veld worden gebruikt, namelijk de standaard sLASER en bewerking-MEGA-sLASER.

We presenteerden daar een interleaved acquisitietechniek die de destructieve factoren moet minimaliseren wanneer acquisities afzonderlijk worden uitgevoerd, zoals B0-inhomogeniteiten, bewegingseffecten en frequentieverschuiving. Het protonenspectrum van GABA bestaat uit drie pieken bij 1,89, 2,28 en 3,01 ppm. De lijnen met 2,28 ppm en 3 ppm zijn gekoppeld aan die met 1,89 ppm. J-coupling, lage concentratie en overlap van de pieken met meer geconcentreerde andere metabolieten maakt het zeer problematisch GABA adequaat te meten met MRS. Echter, terwijl de twee pieken van GABA bij 1,89 ppm en 3 ppm dominant overlappen met andere metabolieten, is 2,28 ppm niet helemaal overlapt, en de dichtstbijzijnde aangrenzende metaboliet is glutamaat bij 2,35 ppm. Omdat het ultrahoge veld profiteert van een grotere scheiding van chemische verschuivingen als gevolg van een toename van de spectrale resolutie, komt de rol van 2,28 ppm GABA in het ultrahoge veld naar voren en is de meting ervan in het ultrahoge veld van belang gebleken. In hoofdstuk 2 hebben we ook aangetoond dat deze GABA-resonantie inderdaad een cruciale rol speelt bij het inschatten van een adequate algemene GABA bij gebruik van aanpassingsmethode LCModel. LCModel vindt een goede aanpassing voor elke metaboliet door minimalisering van de aanpassingsfout en alle GABA-pieken worden meegenomen in het aanpasproces. Als de door LCModel geschatte lijn van 2,28 ppm puur GABA is, helpt het daarom om de adequate en MM-vrije schatting te maken van de 3 ppm lijn in het bewerkte MEGA-spectrum. In **hoofdstuk 3** introduceerden we een nieuwe en uitgebreide versie van sLASER genaamd MASE-sLASER en presenteerden we enkele van de belangrijkste toepassingen. Allereerst hebben we deze sequentie met TE gepresenteerd met een TE van 27 ms, wat vergelijkbaar of lager is dan die van de standaard sLASER die tot nu toe in de literatuur voorkomt. De MASE-sLASER sequentie profiteert bovendien van het kenmerk van gematchte CSDE in alle drie de richtingen die tot nu toe een kenmerk was van een volledige LASER-sequentie en niet van een standaard sLASER-sequentie. Een verbetering in termen van sequentiecomponenten zijn hier MASE-pulsen die semi-adiabatic (excitatie) en adiabatic (heroriëntatie) zijn. Dit resulteert in een lagere gevoeligheid voor B1-inhomogeniteit in vergelijking met de standaard sLASER-sequentie die een conventionele non-adiabatic excitatiepuls heeft. MASE-pulsen kunnen ook korter werken dan de hyperbolische secantpulsen met behoud van een redelijke bandbreedte. Dit gaf de mogelijkheid om de sequentie op korte TE's te implementeren,

ondanks een extra RF-puls in vergelijking met de standaard sLASER-sequentie. De kortere duur van de MASE-pulsen maakte het ook mogelijk om de MEGA-sLASER-sequentie met TE = 68 ms bij 7T te implementeren. Dit is vergelijkbaar met de MEGA-sLASER zoals die in hoofdstuk 2 werd geïmplementeerd met behulp van hyperbolische secantpulsen. Als een andere toepassing van de MASE-sLASER-sequentie toonden we de haalbaarheid aan van het meten van GABA bij 2,28 ppm, goed gescheiden van het aangrenzende glutamaat in deze sequentie met TE = 68 ms bij 7T. GABA bij 2,28 ppm wordt door geen enkele dominante metaboliet overlapt, vooral wanneer het spectrum wordt verkregen in hogere velden waar de hogere spectrale resolutie gunstig is. Het direct meten van deze GABA-resonantie zonder bewerking is interessant en als mogelijkheid gepresenteerd in een eerder werk van Gnaji e.a. met behulp van een lange TE PRESS-sequentie bij 7T. Twee toepassingen van MASE-pulsen die in hoofdstuk 3 worden gepresenteerd, bieden twee verschillende SVS-acquisitiemethoden voor het meten van twee verschillende GABA-resonanties bij dezelfde TE. Een toepassing van deze functie heeft de aanzet gegeven tot het werk dat we in **hoofdstuk 4** hebben gepresenteerd. Met MASE-sLASER bij TE = 68 ms hebben we in hoofdstuk 3 de mogelijkheid gepresenteerd om GABA te meten bij 2,28 ppm, goed gescheiden van het aangrenzende glutamaat. Bij dezelfde TE met MASE-pulsen hebben we ook de MEGA-sLASER-sequentie toegepast. Gebruik makend van de detecteerbaarheid van de 2,28 GABA-lijn in de spectra die met deze twee verschillende technieken werden verkregen, hebben we de haalbaarheid van MM-vrije GABA-metingen aangetoond en hebben we de twee technieken ook onderling gevalideerd. In **hoofdstuk 5** introduceerden we de nieuwe antiphase J verschilbewerkingsmethode. De gedachte achter deze techniek is om de zijpieken te behouden van de GABA-triplet in de antiphase-statussen tussen de twee modi. Door de twee modusspectra ervan af te trekken, worden de centrale piek en de overlappende creatine-singlet geëlimineerd en worden de twee zijpieken in antiphase-status zichtbaar. In theorie werkt deze techniek wanneer de GABA-pieken symmetrisch zijn met exact gelijke koppelingsconstanten, wat in de praktijk niet voorkomt. Derhalve hebben we de oscillatie van het signaal geobserveerd in zowel simulatie- als fantoomresultaten voor lange TE-experimenten. Anders moet de techniek een mono-exponentieel verval laten zien van het bewerkte signaal als factor van TE. De observatie van oscillatie van het antiphase bewerkte signaal, zowel in simulatie- als in fantoommetingen,

werd niet voorspeld in het theoretische perspectief, uitgaande van identieke koppelingsconstanten. We hebben verder onderzoek gedaan met deze nieuwe bewerkingstechniek om het 3 ppm signaal in vivo te meten. Vanwege de tijdsbeperkingen die gelden voor de sequentie en de primaire bewerking was de minimaal haalbare TE zeer lang. De resultaten gaven echter een ander beeld in termen van contaminatie van GABA J bewerkt signaal met MM's. In dit hoofdstuk hebben we de aanwezigheid van lange T2-contaminanten aangetoond, die zelfs bij zeer lange TE's zichtbaar zijn. Dit signaal zou afkomstig moeten zijn van mobiele macromoleculen die vanwege hun mobiliteit lange T2's hebben. Een bewijs voor onze conclusie in dit hoofdstuk was de afwezigheid van het gemeenschappelijk bewerkte 2,28 ppm GABA-signaal in de bewerkte spectra wanneer 1,89 ppm werd geïnverteerd met een zeer smalle bewerkingspuls, wat suggereert dat het bewerkte 3 ppm-signaal geen GABA was.

## Open vragen en toekomstige richtingen

Toekomstig werk met betrekking tot de MASE-sLASER techniek moet onderzoeken of er een TE korter dan 68 ms is waarbij deze sequentie GABA kan meten bij 2,28 ppm, goed gescheiden van het aangrenzende glutamaat bij 7T. Een beperking in termen van GABA meten bij 2,28 ppm met de MASE-sLASER is het lage aantal TE's dat we in dit proefschrift hebben onderzocht, namelijk TE's 68 en 136 ms. Verschillende patronen van glutamaat- en GABA-resonanties in dit gebied zijn een potentiële beperking, omdat deze twee signalen elkaar overlappen, waardoor het onmogelijk is om GABA en glutamaat bij alle TE's te scheiden met de MASE-sLASER-sequentie. Ook het beperkte aantal mogelijke TE's in de MASE-sLASER-methode om GABA bij 2,28 ppm te meten, maakt het moeilijk om T2 van deze GABA-resonantie te meten met deze techniek. In feite wordt ervan uitgegaan dat de T2-relaxatietijd van de twee GABA-resonanties overeenkomstig is om het mogelijk te maken de twee resonantiesignaalintensiteiten te vergelijken zonder T2-correctie. Er zijn momenteel twee onderzoeken in de literatuur die T2 van GABA (3 ppm bewerkt) schatten op 63 ms en 87 ms. Voor T2 van 2,28 ppm GABA is er momenteel echter geen vermelding in de literatuur. Op dit moment hebben we alleen het 2,28 ppm-signaal kunnen meten bij 68 en 136 ms, maar een volledige T2-meting zou meerdere TE's vereisen en de J-coupling van deze resonantie moeten verdisconteren.

De contaminanten die we vonden met lange T2 bij 3 ppm kunnen onderwerp zijn van verder onderzoek. Een mogelijkheid zou zijn om hun relatieve concentratie in witte materie (WM) en grijze materie (GM) in het hersenweefsel te onderzoeken. Er is enig bewijs in de literatuur dat MM's vergelijkbare concentraties in GM en WM hebben. De onderzochte contaminanten met 3 ppm kunnen ook onderwerp van onderzoek zijn om na te gaan of ze kunnen fungeren als kenmerk voor pathogenese. In het algemeen hebben MM's aangetoond dat ze kenmerken zijn voor verschillende aandoeningen, waaronder multiple sclerose (MS) of voor een hersentumor. Het meten van T2 van deze contaminanten kan ook een doel zijn voor toekomstig onderzoek, al kan hun gecompliceerde J-coupling een beperking zijn in deze context.

De antiphase-bewerkingssequentie kan ook in andere lokalisatietechnieken worden geïmplementeerd. De voorwaarde van bewerkingstiming van deze methode biedt de kans om het mogelijk toe te passen op kortere TE's dan de 68 ms vanaf MEGA en dus te profiteren van een hoger SNR. In deze context kan implementatie van de antiphase-bewerkingsmethode in bijvoorbeeld de SPECIAL-sequentie onderwerp zijn van toekomstig werk. De antiphase-bewerkingsmethode kan ook worden gebruikt voor het bewerken van andere J-coupled metabolieten.

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# About the author

Seyedmorteza Rohani Rankouhi (Hani) was born on 22nd January 1983 in Tehran, Iran. After becoming deeply interested in mathematics, physics and chemistry during his education at school, he realised biomedical science was the right topic to study to pursue his academic path. He completed his bachelor's degree in biomedical engineering at Amirkabir University of Technology (Tehran Polytechnic) in Tehran, where he became fascinated by medical imaging. To explore this fascination, Morteza continued his academic studies with a master's degree in medical imaging at the Royal Institute of Technology (KTH) in Stockholm, Sweden. It was during this time that he learned the basics of magnetic resonance imaging (MRI), which he took further on research he performed at the UBC MRI Research Centre in Vancouver, Canada. After completing his master's at KTH, Morteza began his research in Prof. Dr. David Norris' group at the Erwin L. Hahn Institute for MRI in Essen, Germany. His work focussed on magnetic resonance pulse sequence development for GABA spectroscopy, which led to the current thesis. In addition to his professional and academic interests, Morteza enjoys reading, thinking and writing about the philosophy of life, and relishes passing some of his worldly moments writing poems in Persian and English.

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